Second Edition

METHODS IN STREAM ECOLOGY

F. Richard Hauer and Gary A. Lamberti

Editors

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Preface

When the first edition of *Methods in Stream Ecology* was published in 1996, we hoped that it would prove useful to practicing stream ecologists, and perhaps as a supplementary textbook for aquatic ecology courses. However, we and our contributing authors have been delighted that the book has been accepted worldwide as the basic text in stream ecology. The first edition served well for ten years as a reference for both instruction and research. However, as in any dynamic research area, the book was in need of modernization to keep pace with important methodological developments. Unlike the first edition, which stressed exercises that could generally be completed within a few hours or an afternoon of intensive field work, the second edition provides both classroom-style exercises and research-level methods appropriate for the most rigorous investigations.

As we pointed out in the first edition, perhaps no other area of aquatic ecology requires a more interdisciplinary approach than stream ecology. Geology, geomorphology, fluid mechanics, hydrology, biogeochemistry, nutrient dynamics, microbiology, botany, invertebrate zoology, fish biology, food web analysis, bioproduction, and biomonitoring are but a few of the disciplines from which stream ecology draws. The science of stream ecology continues to advance at a remarkably rapid rate, as evidenced by the virtual explosion of publications in stream ecological research during the past two decades. Along with the rapid increase in research activity, we have seen a commensurate increase in the teaching of stream ecology at the upper undergraduate and graduate levels at major colleges and universities. Likewise, scientists, government agencies, resource managers, and the general public have grown keenly aware of stream ecology as an integrative science that can help societies around the globe grapple with environmental degradation of their water resources. Indeed, streams and rivers are fundamental to the human existence, and many organizations and user groups have emerged globally to protect these unique habitats that are so vital to global biodiversity, complexity, and sustainability. We hope that this book will also be of value to these groups.

Stream ecology has experienced many areas of rapidly advancing research, methodologies, and coupled technologies. The serious student or researcher will find that all chapters have been substantially updated and several topics not covered in the first edition have been added with new chapters, notably fluvial geomorphology, nitrogen cycling, dissolved organic matter, fungi, bryophytes and macrophytes, algal biomonitoring, and ecotoxicology. The book continues to provide the most comprehensive and contemporary series of methods in stream ecology, which can be used for teaching or conducting research. We hope that the book will be valuable to both the stream ecology student and the most seasoned scientist. Resource managers employed in the private sector or by federal or state agencies should continue to find this book an indispensable reference for developing monitoring approaches or for evaluating the efficacy of their field and laboratory techniques.

This second edition covers important topics in stream ecology organized within six major sections: Physical Processes; Material Transport, Uptake, and Storage; Stream Biota; Community Interactions; Ecosystem Processes; and a new section on Ecosystem

Quality. Six new chapters have been added to the book, which now contains 36 chapters written by leading experts, and all existing chapters have been substantially revised and updated. Each chapter consists of (1) an Introduction, (2) a General Design section, (3) a Specific Methods section, (4) Questions for the student or researcher, (5) a list of necessary Materials and Supplies, and (6) relevant References. The Introduction provides background information and a literature review necessary to understand the principles of the topic. The General Design presents the conceptual approach and principles of the methods. The Specific Methods generally begin with relatively simple goals, objectives, and techniques and increase in the level of difficulty and sophistication; Basic Methods are suitable for the classroom, whereas Advanced Methods are applicable to high-end research projects. Each method is explained in step-by-step instructions for conducting either field or laboratory investigations. The methods presented are of research quality, and while it is not our intention to produce an exhaustive manual, we present rigorous methods that provide sound underpinnings for both instruction and research purposes. In each case, the methods presented are used frequently by the authors in their personal research or instruction. The Questions listed at the end of each chapter are formulated to encourage critical evaluation of the topic and the methods that were used to address a particular stream ecology issue. The comprehensive list of Materials and Supplies itemizes equipment, apparatus, and consumables necessary to conduct each method and is generally organized by each specific method to allow simple checklists to be made.

If this book is being used for course instruction, we recommend that instructors carefully consider the chapters and methods that they wish to use and plan carefully to budget the necessary time for setup, sampling, and analysis to complete individual or group research projects. Generally, classes should begin with Basic Methods and then delve more deeply into Advanced Methods as time and resources allow. We hope that all of the chapters will enrich the field of stream ecology as a rigorous scientific discipline. As before, we encourage the use of this second edition to assist in the formulation of exciting ecological questions and hypotheses and, to that end, the chapters present sound methods for discovery.

For course instruction, we recommend use of moderate-sized streams from 3 to 12 m wide that are easily waded. Smaller streams should be avoided by a large class, such as 10–20 students, because of the impacts incurred on a small environment. Large rivers are limiting to class instruction because of safety concerns and the inherent difficulties associated with sampling deep, flowing waters.

Reviewers and users of the first edition found this book to be particularly "user friendly." Once again, this was one of our primary goals. As in the first edition, we have attempted to present a book with a logical flow of topics and a uniform chapter format and style, an approach that our authors embraced and implemented. We deeply thank our contributing authors and co-authors from the first edition, who once again gave of themselves and their time for the benefit of our science. We also welcome the authors of the added chapters and likewise thank them for their remarkable efforts. All of them tolerated with (mostly) good humor the fits and starts that characterized the production of this second edition. Chapter reviews were mostly conducted by authors of other related chapters, but several external reviewers also provided us with helpful reviews: Dominic Chaloner, Dean DeNicola, Paul Frost, Brian Reid, Dave Richardson, and Don Uzarski. We are grateful for their assistance.

The inspiration for this book arose from our own research and teaching. Numerous colleagues and students also encouraged the preparation of this second edition, often with suggestions of new chapters or methods that were not treated in the first edition.

We are thankful for their input. Our graduate and undergraduate students continue to be a source of inspiration and encouragement to us even as this book has robbed from our time with them. Our own graduate and postdoctoral advisors (Jack, Art, Vince, and Stan) continue to support our endeavors even as they ruefully concede that "we have become them". We gratefully acknowledge the assistance and financial support of our outstanding home institutions, the University of Montana and the University of Notre Dame. The highly professional staff at Academic Press/Elsevier was a pleasure to work with during this project. Finally, and most importantly, we thank our families for their continued love and support. Our wives, Brenda Hauer and Donna Lamberti, and our children, Andy and Bethany Hauer and Matthew and Sara Lamberti, have energized and inspired us throughout this endeavor and we will be forever grateful to them.

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Section A

Physical Processes

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Landscapes and Riverscapes

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I. INTRODUCTION

Streams, rivers, and groundwater flow pathways are the plumbing of the continents. Water coalesces and flows downhill in surface channels and subsurface pathways in response to precipitation patterns and the dynamic form of river basins (*catchments*). Uplift of mountain ranges, caused by continental drift and volcanism, is continually countered by erosion and deposition (*sedimentation*) mediated by the forces of wind and water. Catchment landscapes are formed by the long geologic and biological history of the region as well as recent events such as floods, fires, and human-caused environmental disturbances (e.g., deforestation, dams, pollution, exotic species).

The term *landscape* is used extensively, referring generally to the collective attributes of local geography. An expansive view of a stream or river and its catchment, including natural and cultural attributes and interactions, is the "riverscape." For a stream ecologist, a riverscape view of a catchment (river) basin encompasses the entire stream network, including interconnection with groundwater flow pathways, embedded in its terrestrial setting and flowing from the highest elevation in the catchment to the ocean, with considerable animal and human modifications of flow paths likely along the way (Fausch *et al.* 2002). For example, the earth's largest catchment, the Amazon River basin, occupies over half of the South American continent. Headwaters flow from small catchments containing glaciers and snowfields over 4300 m above sea level on the spine of the Andes Mountains to feed the major tributaries. The tributary rivers converge to form the mainstem Amazon, which flows from the base of the Andes across a virtually flat plate covered by equatorial tropical forest to the Atlantic Ocean. The altitude change is less than 200 m over the nearly 3000 km length of the mainstem river from the base of

the Andes to the ocean. Because of the enormous transport power of the massive water volume of the Amazon River, some channels are >100 m deep. In other places along the river corridor the channel is >5 km wide, relatively shallow, and filled by sediment deposition (*alluviation*). Flood waters spread out over huge and heavily vegetated floodplains that support a myriad of fishes and other animals (Day and Davies 1986).

The riverscape of the Amazon River, as among all rivers, was molded over time with the river cutting steep canyons through mountain ranges while building (*alluviating*) expansive floodplains where the slope of the river valley decreased. Rivers drain the continents; transport sediments, nutrients, and other materials from the highlands to the lowlands and oceans; and constantly modify the biophysical character of their catchment basins. These processes occur in direct relation to a particular catchment's global position, climate, orography, and biotic character, coupled with spatial variations in bedrock and other geomorphic features of the riverscape.

Within a catchment basin, stream channels usually grow in size and complexity in a downstream direction (Figure 1.1). The smallest or first-order stream channels in the network often begin as outflows from snowfields or springs below porous substrata forming ridges dividing one catchment from another. Two first-order streams coalesce to form a second-order channel and so on to create the network (Strahler 1963). A very large river, like the Amazon, often has several large tributaries, and each of those river tributaries may be fed by several to many smaller streams (Figure 1.1). Thus, each large catchment basin has many subcatchments.

Erosive power generally increases with stream size. Boulders, gravel, sand, and silt are transported from one reach of the stream network to the next in relation to discharge and valley geomorphometry (e.g., slope and relative resistance of substrata to erosion). Expansive deposition zones (floodplains) form between steep canyons, where downcutting predominates.

All rivers feature this basic theme of alternating cut and fill alluviation. Floodplains occur like beads on a string between gradient breaks or transitions in the altitudinal profile of the flow pathway (Leopold *et al.* 1964). Rivers of very old geologic age have exhausted much of their erosive power; mountains are rounded, valleys are broadly U-shaped, and river channels are single threads in the valley bottom with ancient, abandoned floodplains called terraces rising on either side. Whereas, in geologically young, recently uplifted catchments, stream power and associated erosive influence on valley form is great; mountains are steep-sided, valleys narrowly V-shaped, and the river spills out of many interconnected channels on alluvial floodplains in aggraded areas during flooding. Of course, no two rivers are exactly alike, but a general longitudinal (upstream to downstream) pattern of cut and fill alluviation usually exists (Figure 1.1A). Many small and usually erosive streams coalesce in the headwaters to form a main channel that grows in size and power with each primary tributary. The main channel alternately cuts through reaches constrained by bedrock canyons and spills water and sediments onto aggraded floodplain reaches where the river may be quite erosive (cutting) in one place and time and building sedimentary structures (filling) in another; thus, creating a suite of dynamic habitats for biota.

The riverscape at any point within the stream network is four-dimensional (Figure 1.1B). The river continuum or corridor from headwaters to ocean is the longitudinal (upstream to downstream) dimension. The second dimension is the transitional area from the river channel laterally into the terrestrial environment of the valley uplands (aquatic to terrestrial dimension). Except where rivers flow over impervious bedrock, some amount of porous alluvium is present within the channel owing to erosion at
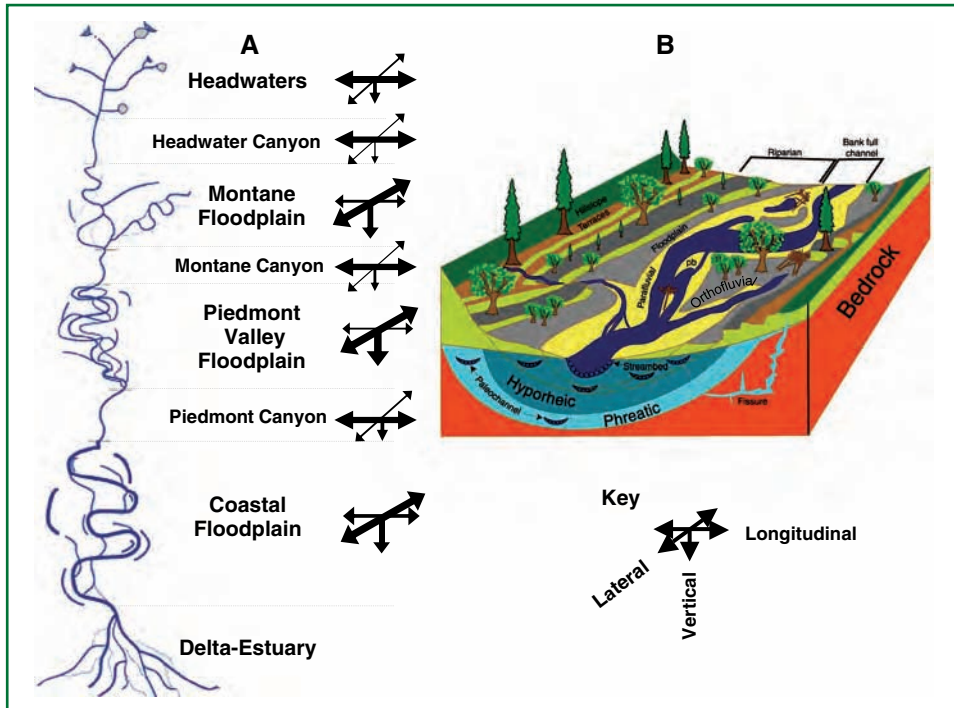
FIGURE 1.1 Idealized view of (A) the stream network showing the coalescence of headwater streams, which begin at snowfields or groundwater discharge portals, and the longitudinal distribution of floodplains and canyons ("beads on a string") within a headwater to ocean river ecosystem and (B) the 3D structure of alluvial floodplains (beads), emphasizing dynamic longitudinal, lateral and vertical dimensions, and recruitment of wood debris. The groups of arrows in (A) indicate the expected strength of ground- and surfacewater exchange (vertical), channel and floodplain (lateral) interactions, and upstream to downstream or longitudinal (horizontal) connectivity in the context of (B). The floodplain landscape, contains a suite of structures (see Table 1.1) produced by the legacy of cut and fill alluviation as influenced by position within the natural-cultural setting of the catchment. The parafluvial zone is the area of the bankfull channel that is to some extent annually scoured by flooding. The hyporheic zone is defined by penetration of river water into the alluvium and may mix with phreatic ground water from hillslope or other aquifers not directly recharged by the river. Alluvial aquifers usually have complex bed sediments with interstitial zones of preferential groundwater flow sometimes called paleochannels. Assemblages of biota may be segregated in all three spatial dimensions including riparos (streamside or riparian), benthos (channel bottom), hyporheos (interstitial within the stream bed-sediments) and phreatos (deep groundwater) in addition to fish and other organisms in the water column of the river (from Stanford *et al.* 2005b).

points upstream. Hence, water from the river may penetrate deeply into the substrata of the river bottom. Moreover, substrata of floodplains are composed of alluvial gravels and/or sands and silts, which allow lateral flow of river water. Hence, interstitial flow pathways constitute a vertical dimension in the river channel and on the floodplains. All of the physical dimensions change in size over time (the fourth dimension), as floods and droughts alter hydrology, sediment transport, and distribution of vegetation and other biota (Ward 1989, Stanford *et al.* 2005b).

Plants and animals are distributed in relation to biophysical gradients expressed by the four-dimensional nature of the stream network within catchment basins. For example, certain species of aquatic insects reside only in the cold, rocky environs of cascading

headwater streams in the high mountains (*rhithron* environments), whereas other species are found only in the much warmer waters of the often sandy, turbid, and meandering reaches of the lowlands near the ocean (*potamon* environments) (Ward 1989). Thus, riverine biota have distinct preferences for specific environmental conditions that are optimal only at certain locations within longitudinal (upstream-downstream), lateral (aquatic-terrestrial), vertical (surface-ground water), and temporal (certain time) gradients that characterize lotic ecosystems (Figure 1.1). Andrewartha and Birch (1954) observed that the essence of ecology is understanding the distribution and abundance of biota. Because environmental conditions at any point in a stream are continuously influenced by conditions at points upstream, biophysical controls on distribution and abundance of riverine biota must be examined in the context of the stream and its landscape setting (Hynes 1975).

A key point is that the riverscape is not static. Rather, it is a dynamic, constantly shifting mosaic or catena of interconnected habitats (Table 1.1) that are created, modified, destroyed, and rebuilt by the interactive processes of cut and fill alluviation mediated by flooding and moderated by riparian vegetation. Trees fall into the channel as powerful flood flows erode floodplain benches covered by forests. The trees obstruct flow, causing deposition of sediments that subsequently allow seedling establishment. Dense growths of young trees catch more sediment, building new floodplain benches and gradually growing into riparian forests. Yet, flooding may knock them down again. Moreover, young riparian trees have to grow fast enough to keep their roots near or in the water table as flooding abates and the volume of the alluvial aquifer declines or they will die. Indeed, the changing volume of the alluvial aquifer and associated rise and fall of the water table coherent with flow in the channel is another important habitat forming process of alluvial floodplains. Overland flooding from the channel to the floodplain is obvious as bankfull flow is exceeded and water spills out of the channel network. Flooding from below ground is less intuitive, but in gravel-bed rivers the initiation of overbank flooding usually is preceded by filling of the alluvial aquifer to the extent that the surface is saturated and hyporheic water erupts into swales and abandoned channels, creating wetlands and spring brooks. Change from dry to wet condition associated with above and below ground flooding is called the *flood pulse*, and it allows aquatic and terrestrial biota to use the same space but at different times, thus vastly increasing biodiversity and bioproductivity of the riverscape (Junk 2005).

The Nyack Floodplain of the Middle Flathead River in Montana is a great example of how plants and animals respond to the flood pulse. This floodplain is very dynamic; the main channel is never in the same place for very long (Figure 1.2). High-resolution remote sensing, coupled with very detailed ground truth studies, have allowed scientists to map the distribution, abundance, and growth of biota within the *shifting habitat mosaic* (*sensu* Stanford *et al.* 2005b) of this floodplain in great detail (Figure 1.3). The parafluvial zone expands and contracts with flooding as cottonwood, willows, and alder generate seedlings during periods of minimal flooding and are washed away during big floods. The orthofluvial zone is built up by deposition of fine sediments, allowing old growth stands of cottonwood and spruce to develop. Wetlands exist in depressions throughout the floodplain, further increasing habitat diversity. Nearly 70% of the vascular plants known in the region occur on this floodplain as a consequence of the shifting habitat mosaic. Other groups of biota are similarly diverse. Thus, the floodplain is in a constant state of change, allowing many species to coexist (Stanford *et al.* 2005b).

To underscore this point, again consider the Amazon. This great river has existed for millions of years, allowing its biota to evolve highly specialized life histories and

TABLE 1.1	Linked Structural (habitat) Elements of Floodplain River Landscapes. Channel and floodplain elements overlap spatially and interact temporally, but time frames differ among rivers. Perirheic habitats are lateral lakes and ponds fed by groundwater that occur on the floodplains of large tropical rivers as described by Mertes (1997). Not all of these elements will necessarily be present on every stream or river because the hydrogeomorphic setting varies even within the same river system (from Stanford *et al.* 2005b).

FLOODPLAIN — entire valley-bottom area that is capable of flooding, including the channel network.

Parafluvial catena — recently reworked by bankfull flooding, usually with driftwood throughout.

Permanently connected channels (eupotamon) — primary, secondary, tertiary channels with characteristic features, such as *thalweg, rapids, shutes, riffles, pools, runs, glides, tailouts, shallow shorelines, channel separation nodes, channel confluence zones, backwaters or side-arms.*

Parafluvial zone — area of annual sediment scour and deposition by floods and wind; with early successional riparian vegetation.

flood channels — seasonally connected overflows.

islands — midchannel areas of sediment accretion, often mediated by wood.

bars and levees — elevated accretion features.

spring brooks (parapotamon) — channelized flow of emergent hyporheic groundwater in flood channels.

ponds or scour holes (plesiopotomon) — perched surface water or emergent hyporheic (groundwater).

Tributary channels — permanent or seasonally disconnected side flows.

Orthofluvial catena — reworked only by big floods or ice jams but frequently inundated, usually with over bank sediment deposits, driftwood, and a riparian vegetation mosaic of age-segregated patches.

Active accretion areas — rapidly enlarging, with variable but mainly well-drained, thin, organic-poor, or variable soils associated with and interfluvial ridge and swale microtopography; may be dissected by flood channels that may contain spring brooks, scour pools, and other parafluvial features.

scrolled (point) bars — continual lateral accretion, usually with woody vegetation precisely age-segregated.

shelves of recent origin — continual accretion, sometimes by driftwood-mediated island aggregation; may have spring brooks, ponds, marshes (paleopotomon) in swales and depressions of abandoned channels and usually dominated by mid-late successional riparian forests or wet meadows.

Passive accretion areas — slowly enlarging, with deeper organically enriched soils associated with swale and ridge microtopography.

shelves of ancient origin — slow accretion associated with extreme flooding into late successional (gallery) riparian forests; may have *spring brooks, oxbows, pans, lakes, billabongs, marshes, bogs* or *muskegs* (paleopotomon) in swales and depressions associated with gradual topographic changes resulting from sedimentation and organic filling of long-abandoned, ancient channels.

floodplain-terrace transition zone — mixed upland and floodplain vegetation; often with spring brooks or wet lands in wall-based flood channels.

TERRACES — ancient floodplains, disconnected from the active floodplain system by incision and no longer inundated by floods; dominated by terrestrial vegetation.

HILL SLOPES (VALLEY WALLS) — terrestrial, but may be substantially influenced by microclimatic influences of the floodplain; may have slope wetlands from with disjunctive floodplain vegetation.

[left margin, vertical: Increasingly terrestrial]

[left margin, vertical: Perirheic habitats]
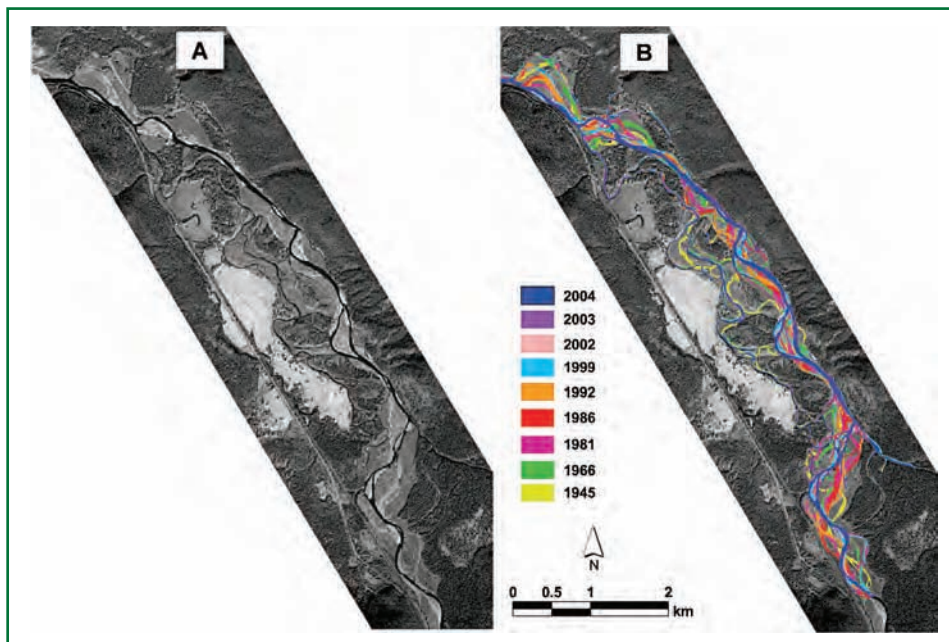
FIGURE 1.2 (A) A satellite multispectral image of the Nyack Floodplain habitat catena (Middle Flathead River, Montana 2004). Most, but not all, elements given in Table 1.1. are found on this floodplain. The floodplain extends laterally to both valley walls and is essentially a bowl filled with gravel and rock with a thin veneer of fine sediment and gradually developing soils on the higher-elevation benches that are not scoured by flood flows. Much of the gallery forest of cottonwood and spruce has been cleared for hay farming. Owing to the porous nature of the valley bedsediments, a legacy of river deposition since glaciation, river water downwells into the alluvial aquifer beginning at the upstream knickpoint where the river becomes unconstrained by bedrock. The downstream knickpoint defines entry into another bedrock-constrained canyon, which impounds the alluvial aquifer, allowing it to intersect the surface creating spring brooks and wetlands as water flows from the aquifer back into the river. (B) Here the position of the main channel during 1945–2004 has been color coded to emphasize the dynamic nature of the river (Flathead Lake Biological Station, unpubl. data).

morphologies in response to long-term dynamics of the river environment. Seemingly countless aquatic and semiaquatic species coexist, each trying, and variously succeeding, to grow and reproduce in accordance with evolved life history traits and within the myriad of environmental gradients expressed by the dynamic course of the river through the massive catchment basin. For example, the adaptive radiation of Amazonian fishes is astounding, ranging from deep-water specialists that reside in the dark depths of the scoured channels to species that reproduce exclusively in the floodplain forests during floods (Junk *et al.* 2000, Lowe-McConnell 1987, Petrere 1991). Perhaps even more profound are interpretations of satellite-derived images that strongly suggest the enormously complex and highly evolved rain forests of the Amazon Basin are composed of a mosaic of successional stages created by the river cutting and filling its way back and forth across this huge landscape century after century (Colinvaux 1985, Salo *et al.* 1986).

We can conclude from studies on the Amazon and many other river systems that the first task of a river ecologist is to determine the appropriate scale of study to answer any particular question at hand (Poole 2002). Do I need to examine the problem in the
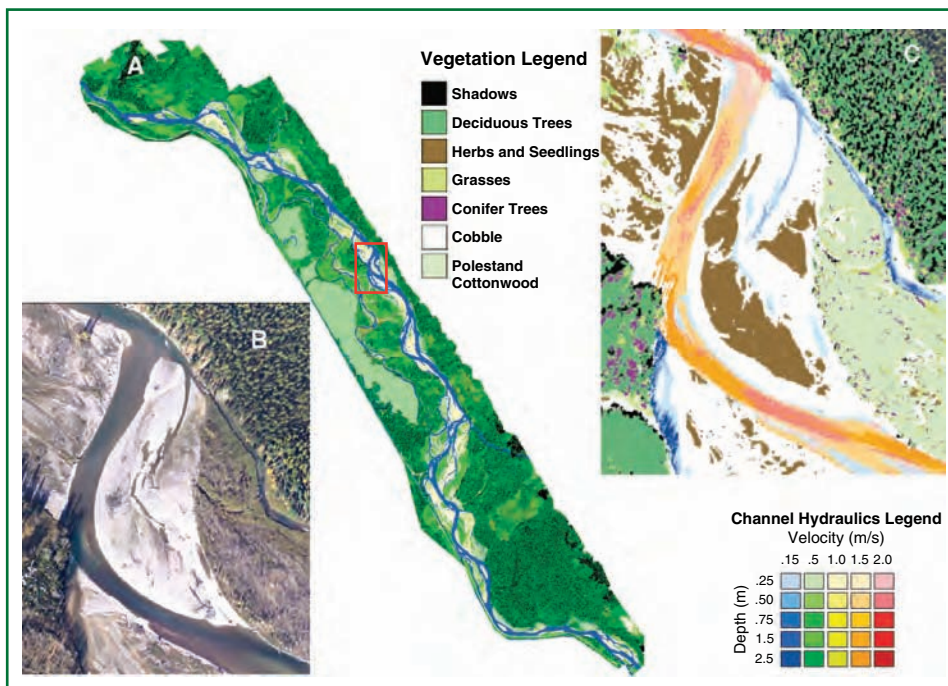
FIGURE 1.3 (A) Hyperspectral data for the image in Figure 1.2.A. of the Nyack Floodplain have been classified into various vegetation types (1 m resolution) using geographical information software (GIS) and validated using ground truth surveys. Inset (B) is a high-resolution digital image that has been classified in (C) showing the distribution of depth and velocity of the water in the image along with the same vegetation types as in (A). Classification of such attributes allows spatially explicit determination of the distribution and abundance of floodplain habitat used by fishes and wildlife (Flathead Lake Biological Station, unpubl. data).

context of the entire river continuum from headwaters to the ocean or will a particular reach or even a particular riffle or pool suffice?

Moreover, this dilemma of spatial scale is complicated by the fact that the full range of biophysical features of rivers may change suddenly as a consequence of intense, unusual events like very large floods, extended droughts, catchmentwide fires, earthquakes, volcanic eruptions, and other natural phenomena that may radically change conditions reflected by the long-term norm (Schumm and Lichty 1956, Stanford *et al.* 2005b). So what time period needs to be encompassed by a study in order to adequately understand the ecological significance of natural disturbance events?

And, of course, natural variation in time and space is superimposed upon environmental change induced by human activities in catchment basins. Native people have always been a part of riverscapes worldwide, shaping it to their needs by diverting flows for irrigation of crops or increasing wetland plants, burning forests to increase berry shrubs and harvesting riverine biota. Native societies simply moderated the shifting habitat mosaic, whereas modern societies have vastly altered the process. Flows in all of the larger and most of the smaller rivers in the temperate latitudes of the world now are regulated by dams and diversions, and the tropics are under siege (Dynesius and Nilsson 1994, Nilsson *et al.* 2005). Reduced volume and altered seasonality of flow radically change the natural habitat template, eliminating native species and allowing invasion of nonnatives.

In many cases water from dams is discharged from the bottom of reservoirs, drastically changing temperature patterns and armoring the river bottom by flushing gravel and sand and leaving large boulders firmly paving the bottom. Problems related to flow regulation in other cases are exacerbated by pollution and channelization (Petts 1984). A wide variety of other human effects can be listed (Table 1.1). The cumulative effect is the severing or uncoupling of the complex interactive pathways that characterize the four-dimensional shifting habitat mosaic of riverscapes. Generally, the result is a vastly less dynamic environment than occurred naturally that substantially compromises biota that are adapted to the shifting habitat mosaic often allowing invasion of nonnative, noxious species (Stanford *et al.* 1996).

II. GENERAL DESIGN

A. Analysis at the Riverscape Scale

The purpose of this chapter is to provide a riverscape or ecosystem context for the other chapters in this book, which teach detailed and more site-specific analyses of river ecological processes and responses. The premise is that few river research and management questions can be answered without considering riverscape attributes and dynamics and very often, issues outside the catchment basin may also be very important. Indeed, almost all natural resource management questions have to be addressed in a whole basin or river ecosystem context owing to overlapping jurisdictions and the interactive nature of ecological processes that provide riverine goods (clean water, fisheries, wildlife) and services (transportation, water power, floodplain fertilization) that humans require. However, ecosystem boundaries are permeable with respect to energy and materials flux and often are best determined by the nature of the ecological issue or question of concern (Stanford and Ward 1992).

Consider the problem of conserving wild salmon around the world. Wild salmon have steadily declined worldwide due to overharvest and vastly altered habitat conditions in many of their natal rivers. For example, at least 20 million Pacific salmon and steelhead historically returned to the Columbia River to spawn. Over 200 different runs (populations) of the five most abundant species occurred as a result of thousands of years of adaptation to the shifting habitat mosaic and the high productivity of the very complex riverscape of this huge ($567,000\,km^2$) catchment basin. Adult salmon returned with great fidelity to specific sites along the entire river corridor, including headwater streams and lakes in many of the tributaries (e.g., Snake River Subbasin). Juveniles moved around a lot, often focusing on floodplain habitats and grew on the rich food sources provided by the shifting habitat mosaic. Populations of some species stayed in the river only a few months, while others stayed for three to five years. This same life history plasticity occurred in the ocean with some returning to spawn after only one or two years, while others such as the huge Columbia River chinook (locally called June hogs for the timing of the run and their $25\,kg+$ body size) fed for several years in the ocean before returning. This life history plasticity underscores the ability of salmon species to adapt their life cycle to local conditions encountered. The Columbia River encompasses 15 ecoregions, thus presenting many different shifting habitat venues for salmon to use, and the result was a very high diversity of locally adapted populations. Entrainment of salmon carcasses in the riparian zone increased fertility (salmon die after spawning, steelhead do not) in addition to providing food for a wide variety of wildlife and humans living along the

river and its many tributaries. Thus, the predevelopment Columbia was a natural salmon factory that supported thousands of native people (Stanford *et al*. 2005a, Williams 2006).

Of course, today the Columbia River and almost all of its tributaries are completely harnessed by hundreds of dams that vastly alter natural flow patterns. Almost the entire mainstem is impounded by hydropower operations making salmon migration problematic. Floodplains either are flooded by reservoirs or severed from the river channel by highways, railroads, and encroaching towns and cities. Farms and industries of all sorts have been developed by diverting water from the tributaries and the mainstem. Billions of dollars have been spent to recover salmon in the Columbia River since 1990 to little avail. Indeed, only one really robust run remains, fall chinook that spawn and rear to smolts (ocean going juveniles) in the Hanford Reach, the last free flowing stretch of the mainstem river (Stanford *et al*. 2005a, Williams 2006).

The situation is not much better in most of the other salmon rivers of the world, except those in the far north where the shifting habitat mosaic remains unaltered by human activities. Salmon have been lost altogether in most European Rivers such as the Rhine River where they were once abundant.

But owing to their iconic and economic status in local cultures along rivers where salmon were once abundant, people want the salmon back and are willing to pay for it. The challenge for river ecologists is how to allow use of the rivers for the full array of human demands and at the same time provide habitat for salmon. At least two main issues must be considered. First, one has to realize that the ecosystem of the salmon includes the entire river system as well as its estuary and a large area of marine environment, and that for Pacific salmon by example, means most of the North Pacific Ocean where they migrate from one feeding area to another depending on ocean conditions. So far, managers have not tailored harvest of salmon to account conservatively for variation in ocean, estuary, and riverine conditions that salmon will encounter during their long life cycle, and the result has been years of overkill that has eventually reduced the runs to the point that they cannot be sustained in spite of their natural plasticity to environmental variation. Second, the shifting habitat mosaic that salmon require in freshwater has to be provided by restoring normative flow to the natal rivers (Stanford *et al*. 1996, Hauer *et al*. 2003, Hauer and Lorang 2004, Stanford *et al*. 2005a). In many systems, like the Columbia, this can only be reasonably done on certain tributaries that do not have large hydropower dams and other infrastructures that people are not willing to give up in spite of general favoritism for salmon. Other issues such as climate change and use of hatcheries to mitigate lost habitat also are problematic (for more information about salmon, see www.wildsalmoncenter.org).

On a smaller scale, but with equally interactive research and management issues in river ecology, is the Flathead River-Lake ecosystem in northwestern Montana and southeastern British Columbia, Canada (Stanford and Ellis 2002). It is a large ($22,241 \, \text{km}^2$) subcatchment of the Columbia River that was historically unavailable to salmon owing to natural barrier falls that prevented upstream migration. The catchment encompasses small urban and agricultural lands on the piedmont valley bottom, extensive national (US) and provincial (BC) forests with forest production and wilderness management zones, and the western half of the Glacier-Waterton International Peace Park, an International Biosphere Reserve and World Heritage Site. The altitudinal gradient extends some 3400 m from the highest points on the watershed to Flathead Lake. Indeed, the flow of the river begins on Triple Divide Peak, the crown of the continent where three of the great rivers (Columbia, Saskatchewan, and Missouri) of North America begin. The riverscape of the Flathead is multifaceted, with crystal mountain streams cascading through steep mountain valleys

that contain abundant populations of Rocky Mountain wildlife, including one of the two last populations of grizzly bears in the United States (the other is in the Greater Yellowstone Ecosystem). The river system includes the Nyack Floodplain just described, where the concept of the shifting habitat mosaic was developed and other expansive floodplains along each tributary and the main stem in the Flathead Valley that flows into $480\,\text{km}^2$ Flathead Lake, the largest lake in the western United States and among the cleanest in the world for large lakes that have significant human populations in the catchment basin.

But the Flathead is under the same pressures that most river and stream systems endure. The limnology of Flathead Lake and its river system has been studied for over 100 years by scientists at the Flathead Lake Biological Station of the University of Montana. This record clearly shows that water quality is gradually declining in direct relation to development of the human infrastructure in the basin. In recent decades, the decline in water quality has accelerated in response to very rapid population expansion. Driven by strong desire to sustain high water quality, particularly the amazing transparency of Flathead Lake, and the very clear demonstration of change for the worse by the scientists, citizens of the Flathead supported construction of modern sewage treatment plans throughout the catchment and effectively implemented land use regulations to minimize diffuse runoff of pollutants from cities, farms, and logging operations. This is a success story for river management, although continued vigilance and careful river and lake monitoring is required.

However, a continuing major problem for river ecologists in the Flathead is the change in the food web structure of Flathead Lake. Species of fish and invertebrates were introduced to increase fishing opportunities, actions that were scientifically uninformed. Indeed, the abundant native trout, the cutthroat, began to decline when landlocked red salmon (kokanee) were introduced, along with lake trout, lake whitefish, bass, and other species. The native predatory fish, the bull charr, adapted to feeding on kokanee as their native prey, the cutthroat, declined. Bull charr (and cutthroat trout) migrate from the lake to specific tributaries to spawn. Juveniles stay in the river system for several years before migrating to the lake to mature. Thus, the life cycle is rather like salmon in that the lake and river system encompass the bull charr's life history ecosystem. Bull charr persisted happily in Flathead Lake until mysid shrimp (*Mysis relicta*) were introduced with the thought of increasing productivity of kokanee through added mysid forage. The introduction backfired badly because it turned out that the mysids ate the food of the kokanee, but the kokanee could not in fact forage on mysids because the mysids were only active at night, whereas kokanee are daytime feeders. This effectively eliminated the formally abundant kokanee from the lake. At the same time the mysids provided abundant new forage for lake trout and lake whitefish that were previously impoverished by having poor food resources for early life stages and were only very slowly expanding. The new mysid forage was ideal for juvenile lake trout and lake whitefish and allowed these species to expand rapidly at the expense of the bull trout, which declined precipitously. Today the lake is dominated by nonnative fish. The native bull charr, cutthroat, and other native fishes are in danger of extirpation from the system. In addition, the now abundant nonnative species have emigrated upstream and colonized lakes in Glacier National Park, presenting yet another dilemma for river managers. The Federal Endangered Species Act and the charter of the National Park system in the United States require conservation and protection of native species. This will be decidedly problematic in the Flathead, owing to seemingly irreversible food web changes promulgated by past management mistakes.

These examples underscore the importance of understanding river and stream systems in a riverscape ecosystem context. A clear definition of the ecosystem boundaries that influence environmental problems is required. The bottom line is that today's stream ecologist must be broadly trained, attuned to a multidisciplinary, riverscape approach to problem solving, and fully informed scientifically to do the job right.

III. SPECIFIC METHODS

A. Basic Method 1: Boundaries and Hydrography of the Catchment Basin

Catchment boundaries are the ridges that separate a catchment basin from those adjacent. Technically, the catchment boundaries should be termed *watersheds*. However, in the United States *watershed* often is considered synonymous with *catchment basin*. The hydrography (spatial distribution of aquatic habitats) of a catchment basin can be conveniently examined at 1:24,000 scale using maps available from the United States Geological Survey.

1. Using larger-scale maps of your research area, determine catchment basin boundaries for a region of at least $10,000\,km^2$. Choose one catchment of at least $100\,km^2$ area for detailed examination. Using a planimeter, determine the total area of the basin.
2. Note the stream network, shown in blue on most maps. Compare the detail of the catchment on different scale maps. The smallest streams begin at higher elevations (e.g., snowfields, lakes, wetlands, or springs). Groundwater aquifers often erupt from hillsides via upslope infiltration of precipitation through porous soils or bedrock. In many cases the smallest stream channels are shown as broken lines, which indicate that surface flow is intermittent.
3. If many intermittent stream channels are shown, the catchment basin is either very dry or the substrata are very porous. In both mesic (wet) and xeric (dry) landscapes, a large amount of the runoff may follow subterranean (groundwater) pathways through porous substrata (see Stanford *et al.* 2005b). Differentiate intermittent and permanent stream channels in your catchment.
4. An important point to keep in mind is that the drainage network really is a geohydraulic continuum; that is, the stream corridor has both surface and groundwater components, and these interactive pathways are hydrologically and ecologically interconnected (Gibert *et al.* 1994). Water flowing at the surface at one place may be underground at another, depending on the geomorphology of the catchment basin and the volume and timing of rainfall or snowmelt. Hence, interaction zones between surface and groundwaters are fundamental attributes of landscapes and are very germane to stream ecological studies. Compare topographic, geologic, and groundwater maps of your catchment and identify potential areas of near surface ground waters that may be fed by surface waters or discharge into the stream network.
5. Stream order is determined by the coalescence pattern (see Figure 1.1 and Chapter 4, Figure 4.1). Two first-order streams converge to form a second-order stream, two second-order tributaries form a third-order stream, and so on. Network density is related to geologic origin of the basin, time since uplift, precipitation patterns, precipitation history, types of vegetation present, and

resistance of substrata to erosion and infiltration. Lay out a series of maps covering the study catchment at 1:24,000 scale. Overlay the maps with clear plastic or acetate sheets. On the plastic sheets, color-code the different stream orders with markers and tabulate them on a data sheet. Measure length of all streams within the catchment, using a map wheel. Simply trace the stream corridor with the map wheel starting at zero and reading the distance on the appropriate scale of the wheel. Calculate drainage density of the catchment as total stream length divided by total area of catchment.

6. Observe the altitudinal gradient from highest to lowest elevation in the catchment. Carefully consider the density of topographic isopleths (lines of equal elevation). Where they converge closely adjacent to and across the stream channel, canyon segments exist. More widely spaced isopleths indicate flatter topography. Use the acetate sheets overlaying the topographic maps set up in step 5 to locate gradient breaks.

7. Identify canyons (downcutting channels confined by bedrock walls) and alluvial (aggraded, unconfined channels with wide, terraced floodplains) stream segments. In many cases alluvial deposits will be shown by special designations on the maps. Check the map key for such designations. In alluvial zones you may observe that the stream channels begin to braid, which suggests major deposition and floodplain development. On alluvial reaches of bigger streams, the general structure of the floodplains will likely be evident in the form of active zones of flood scour and terraces at higher elevations along the channel. Using elevation data from the topographic maps, plot the stream profile from highest to lowest elevation (x-axis = distance downstream; y-axis = elevation). Label the major gradient breaks and alluvial reaches.

8. Streams may flow into lakes or wetlands. In some cases wetlands may remain where lake basins have filled with sediments. In glaciated landscapes, lakes may occur in high-altitude cirques; larger, often very deep lakes may occur singly or in a series in the glaciated mountain valleys. Many lakes and wetlands are fed and drained by groundwater and determination of underground flow pathways may require geohydrologic surveys. Consult Wetzel (2001) for detailed descriptions of the types of lakes and modes of origin. The main point here is to note the position and potential influence of lakes on the stream network of your catchment basin. Lakes function as sinks for fluvial sediments, nutrients, and heat. Streams flowing from lakes may well be very different than inflowing streams. Manmade reservoirs function in similar fashion, except that ecological influences on rivers below the dams will depend on the depth and mode of water release from the dams (Stanford *et al*. 1996, Poff *et al*. 1997). Tabulate lakes and reservoirs in your catchments, noting elevation, area, and other available data (e.g., volume, flushing rate).

B. Basic Method 2: Other Landscape Attributes of the Catchment Basin

The maps provided likely will show surficial geology, groundwater resources, broad vegetation categories, precipitation patterns, and human infrastructures (roads, pipelines, dams, railroads, urban areas, or individual buildings, etc.). Systematic summarization of these features in relation to the hydrography will provide valuable insights about potential influences on water quantity and quality and constraints on distribution and abundance of riverine biota. For example, an understanding of the general geology of

the catchment basin will provide insights into discharge, water chemistry, distribution of biota, and other attributes of the catchment basin that likely will be encountered in fieldwork. Igneous and metamorphic rocks generally do not dissolve much in water, and, hence, surface waters draining such formations have low dissolved solids and little buffer capacity, whereas waters from limestone formations generally may be expected to contain high amounts of dissolved solids and be very well buffered.

Land use patterns inferred from the distribution of human infrastructures shown on the maps can be corroborated from aerial photographs and satellite images. Google Earth and other Internet map tools allow a quick view of the riverscape (http://earth.google.com/). If the photos are available in a time series, changes in hydrography (e.g., channel migration on floodplains) as well as changes in land use patterns can be observed.

1. Using a map wheel and planimeter for the maps (may use digitizing tablet and computer if available) and a stereoscope for aerial photos of known scale, determine the lengths and areas of various features on the landscape of the river catchment you have chosen for study. Create a table or computer spreadsheet in which you can record the different landscape attributes identified in the steps below. Record the features by stream length, area, or other spatial measures. This will provide a basis for a general description of the study catchment and landscape attributes that may influence ecological processes and responses within the stream network.

2. Compare the catchment basins you have identified on the topographic maps with geologic maps of the region. On granitic and other "hard rock" mountains, runoff usually is dominated by surficial flow, whereas limestone and other sedimentary and volcanic formations may allow considerable infiltration and runoff may predominately follow groundwater pathways to portals back to the surface at lower elevations. Subsurface drainage networks dominate in karst (cavernous limestone) landscapes (see Mangin 1994). Tabulate the major geologic formations by type and percent of catchment basin area. Use a planimeter to determine areas of different geologic formations.

3. Determine vegetation cover patterns within the catchment basin. At a minimum the topographic maps should show forest or grassland areas in green and exposed bedrock or other nonvegetated (e.g., clear-cut forest stands) areas in white. Glaciers and wetlands likely will have special designations shown in the map key. Vegetation maps of your catchment may be available or you may be able to use aerial photos to determine the general pattern in comparison to the topographic maps. Using all available maps and photos determine at a minimum riparian (stream side), wetland, and upland (forest and grassland) ground cover for the entire catchment basin. For montane regions it is instructive to differentiate forest types with respect to altitude (e.g., riparian, upland forest, subalpine forest, alpine). Again use the planimeter to determine areas of cover types and record percent of basin area by type.

4. Examine the stream corridors on the topographic maps for features created by human activity, such as revetments, bridges, irrigation diversions or returns, mines, and other industrial sites. All of these may change flow patterns or otherwise influence the natural attributes of the stream corridor. On the acetate sheets, color-code stream segments by type of alteration or land use. Tabulate percent of stream corridor and/or catchment basin potentially influenced.

5. If aerial photos are available, verify all the features you have identified in the catchment basin(s) from interpretation of maps. Add notes for features more

evident in the photos, such as riparian forests or stream channels. Can you identify a subset of the habitats given in Table 1.1? Keep in mind that the maps and photos may have been produced on very different dates and therefore show differences in the landscape features.

6. Note any discharge or precipitation gauging stations in your catchment basin. These are sometimes included on topographic maps. Prepare time series plots of available data for these stations and calculate unit area precipitation and runoff. Determination of stream flow is discussed in more detail in Chapter 3, but knowing stream flow dynamics at various points in the stream network will provide a more complete view of the catchment landscape as derived in this chapter from maps and photos.

C. Advanced Method 1: Computerized Spatial Analyses of Riverscapes

While maps and photos are basic tools for understanding how your study basin fits into the regional landscape, digital approaches provide a means for examining landscapes in great detail. All points in any landscape can be precisely known from geodetic surveys. In fact, that is how the topographic maps used above were created. With the aid of a computer, topography can be reduced to a digital data base using algorithms that interpolate between surveyed points. Using software that is widely available, the computer operator can produce three-dimensional images of any digitized landscape. Topographic data can then be examined statistically or plotted in relation to any other spatial data bases (e.g., stream network, water quality, fish distribution).

A number of software packages that manipulate digitized data in relation to geographic references are available under the general descriptor of geographic information systems (GIS). Considerable computer sophistication is required to use a GIS properly, although most can be run on high-speed personal computers. The advantage of a GIS is that landscape data for many variables can be created in "layers" superimposed in relation to the topography (Figure 1.3). This is a very useful way to accurately keep track of and display landscape change over time. For example, observed fish distributions within a catchment basin can be plotted in true spatial (geographic) context with the hydrography and, if time series data are available, changing fish distributions can be shown in spatial relation to changes in potentially controlling variables, such as land use activities. Hence, a GIS permits very large data sets to be systematically arrayed and related in time and space in a manner that facilitates interpretation of landscape pattern and process (Bernhardsen 2002, Longley *et al.* 2005).

Moreover, data describing landscape patterns in some cases can be derived from spectral (reflectance) data gathered from satellite or other "remote" sensors. In this case a GIS is essential to relate massive amounts of spectral data for entire landscapes to the actual topography. Different wave lengths of light are reflected by the pattern of landscape attributes on the ground. Hence, algorithms or statistical models can be derived that relate measured spatial variation for a portion of the landscape (ground truth data) to the variation in the spectral patterns recorded remotely. The algorithms then can be used to generate landscape data layers in direct relation to the topography (Lillesand and Klefer 2000). Obviously, some landscape variables are better suited to spectral imagery than others, and considerable ground truthing is needed to verify the accuracy of the remotely sensed data. For example, water bodies are easily distinguished from terrestrial environs; coniferous forests can be distinguished from grasslands. But

this technology is in a rapid state of development and should be approached with caution and a clear understanding of the research or management question.

Most research universities have spatial analysis laboratories. If a GIS is not available to demonstrate utility in landscape analysis of catchment basins, I recommend that an active spatial analysis lab be toured to clearly convey the usefulness of this technology in demonstrating pattern and process at the level of entire catchment basins.

D. Advanced Method 2: Identifying Ecosystem Problems at the Landscape Scale

Now that you have summarized landscape features of your catchment basins, it is important to consider what sorts of questions or problems require resolution at a landscape scale. Almost all natural resource management questions have to be addressed to some extent in a landscape context, owing to overlapping jurisdictions. For example, in the Flathead catchment just described, nearly all federal (US) land management agencies (e.g., Forest Service, Environmental Protection Agency, Bureau of Reclamation, National Park Service) and a wide variety of state and tribal agencies have legislated authority for water resources. Without a landscape perspective, one agency could easily initiate a management objective that interferes with actions of another agency. Moreover, the ability of ecosystems to provide ecological goods and services (e.g., water, timber, wildlife, scenery) to humans clearly encompasses local to regional landscapes.

But, to resolve specific problems, is it necessary to study the entire catchment? If so, how big should the catchment be? Are catchment boundaries also ecosystem boundaries? All are difficult questions. Properly scaling the research and management approach perhaps is the most difficult task faced by any ecologist. However, thorough synthesis of available information about the particular problem in a landscape context is the place to start.

In the case of the Flathead Lake bull charr, the entire catchment of the Flathead River clearly is the ecosystem that sustains this fish. In this case the catchment boundaries are the ecosystem boundaries in this very large and complex landscape. For salmon the ecosystem boundaries are much larger, extending beyond the river catchment well into the ocean. Determining the causes and consequences of the bull charr and salmon declines and implementation of a solution to sustain fisheries over the long term must involve an understanding of the habitat requirements of the various life history stages of the fish as well as a predictive understanding of the complex biophysical processes that control the quantity and quality of those habitats.

River ecosystems encompass ecological, social, and economic processes (ecosystem functions) that interconnect organisms (ecosystem structure), including humans, over some time period. The ecosystem boundaries are permeable with respect to energy and materials flux; therefore, even large systems are influenced by external events such as global climate change, pollution, national and global economies, and emigration of people and nonnative biota. This holistic view should be kept in mind as approaches to resolution of river ecological questions are considered.

1. Using the salmon example as a general guide, list a series of river ecological questions that may be inferred from the landscape attributes of your catchment basins. For example, if your catchment is dominated by agricultural lands, what sorts of river problems might you expect?
2. Determine where in your catchment basin you would place monitoring or study sites to assemble river ecological information to solve your list of problems.

IV. QUESTIONS

A. Boundaries and Hydrography of the Catchment Basin

1. Based on your measures of the stream network, does it appear that your catchment basin is very dry or very wet?
2. How does "wetness" of the catchment basin relate to the density of the stream network?
3. Is the stream network "fragmented" in any way by natural obstructions, such as landslides, lakes, wetlands, beaver dams, or interstitial flow pathways, or is the stream profile a continuous surface flowpath?
4. Are alluvial floodplains a dominant feature of the stream corridor?
5. Is the floodplain habitat catena intact?
6. Is discharge from the catchment likely to be significantly controlled by reservoir storage and/or dam operations?

B. Other Landscape Attributes of the Catchment Basin

1. Is the bedrock substrata of your catchment basin likely to be very porous or will most of the precipitation run off via surficial channels?
2. Is the water chemistry of the river system likely to be well buffered or poorly buffered?
3. Is your catchment basin human dominated?
4. What manmade structures are present in the catchment basin that might change flow and channel form or obstruct migrations of biota?
5. What stream channels appear to have a closed canopy as a consequence of dense riparian forest and how might canopy cover influence ecological processes in the stream?
6. What is the predominate vegetation type in the catchment basin and how might it influence river ecology?
7. What is the predominate human land-use activity in the catchment basin and how will that activity likely affect river ecology?

C. Computerized Spatial Analyses of Landscapes

1. What sorts of problems in river ecology do not require use of a computerized GIS system?
2. What problems in river ecology might usefully be analyzed by a computerized GIS in your catchment?
3. How can a GIS be used to document landscape changes in your catchment basin?

D. Identifying Ecosystem Problems at the Landscape Scale

1. Have you included all landscape units in your catchment basin that may exert significant ecological influences on the river system?
2. Can you observe fragmentation in geohydraulic continuum caused by human activities and, if so, how do they relate to your list of research and management problems?

3. Are your catchment boundaries also ecosystem boundaries with respect to your list of river ecological problems?
4. Based on the information about bull charr and the landscape attributes of the Flathead catchment described, answer the following questions:
    a. What processes likely are influencing the distribution and abundance of bull charr in the Flathead River-Lake catchment?
    b. State these likely processes in the form of hypotheses about the decline of Flathead Lake bull charr.
    c. What landscape information is needed to test these hypotheses?
    d. What other ecological information and data are needed to better understand the bull charr problem and in what time frame should these data be collected?

V. MATERIALS AND SUPPLIES

Aerial photo series, preferably stereo pairs, and/or multispectral digital image data
Geologic maps
GIS demonstration
Groundwater maps
Map measurement wheels
Planimeters or digitizing pads and computers
Plastic overlays and color markers
Stereoscopes for photo interpretation
Topographic maps of the study region at various scales
Vegetation maps

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Valley Segments, Stream Reaches, and Channel Units

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I. INTRODUCTION

Valley segments, stream reaches, and channel units are three hierarchically nested subdivisions of the drainage network (Frissell *et al.* 1986), falling in size between landscapes and watersheds (see Chapter 1) and individual point measurements made along the stream network (Table 2.1; also see Chapters 3 and 4). These three subdivisions compose the habitat for large, mobile aquatic organisms such as fishes. Within the hierarchy of spatial scales (Figure 2.1), valley segments, stream reaches, and channel units represent the largest physical subdivisions that can be directly altered by human activities. As such, it is useful to understand how they respond to anthropogenic disturbance, but to do so requires classification systems and quantitative assessment procedures that facilitate accurate, repeatable descriptions and convey information about biophysical processes that create, maintain, and destroy channel structure.

The location of different types of valley segments, stream reaches, and channel units within a watershed exerts a powerful influence on the distribution and abundance of aquatic plants and animals by governing the characteristics of water flow and the capacity of streams to store sediment and transform organic matter (Hynes 1970, Pennak 1979, Vannote *et al.* 1980, O'Neill *et al.* 1986, Statzner *et al.* 1988). The first biologically based classification

| TABLE 2.1	Levels of Channel Classification, Each with a Typical Size Range and Scale of Persistence. After Frissell *et al.* (1986) and Montgomery and Buffington (1998).		
Classification Level		**Spatial Scale**	**Temporal Scale (years)**
Channel/Habitat Units		$1–10\,m^2$	$<1–100$
Fast water			
Rough			
Smooth			
Slow water			
Scour pools			
Dammed pools			
Bars			
Channel Reaches		$10–1,000\,m^2$	$1–1,000$
Colluvial reaches			
Bedrock reaches			
Free-formed alluvial reaches			
Cascade			
Step-pool			
Plane-bed			
Pool-riffle			
Dune-ripple			
Forced alluvial reaches			
Forced step-pool			
Forced pool-riffle			
Valley Segment		$100–10,000\,m^2$	$1,000–10,000$
Colluvial valleys			
Bedrock valleys			
Alluvial valleys			
Watershed		$50–500\,km^2$	$>10,000$
Geomorphic province		$1,000\,km^2$	$>10,000$
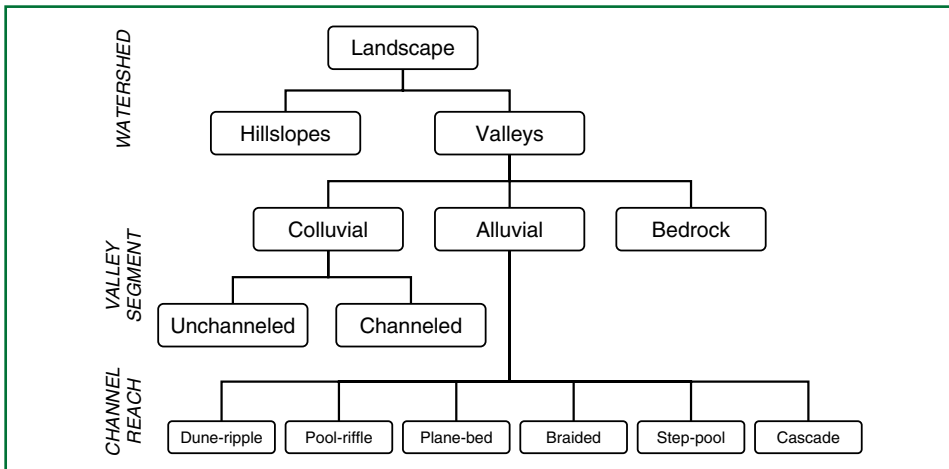
FIGURE 2.1 Hierarchical subdivision of watersheds into valley segments and stream reaches. After Montgomery and Buffington (1997).

systems were proposed for European streams. They were based on zones marked by shifts in dominant aquatic species, such as fishes, from a stream's headwaters to its mouth (Huet 1959, Illies 1961, Hawkes 1975). Characterizations of biologically based zones have included the effects of physical processes and disturbance types on changes in faunal assemblages (Zalewski and Naiman 1985, Statzner and Higler 1986). Hydrologists and fluvial geomorphologists, whose objectives for classifying streams may differ from those of aquatic biologists, have based classification of stream channels on topographic features of the landscape, substrata characteristics, and patterns of water flow and sediment transport (Leopold *et al.* 1964, Shumm 1977, Richards 1982, Rosgen 1994, Montgomery and Buffington 1997, Montgomery and Bolton 2003). Other approaches to classifying stream types and channel units have combined hydraulic or geomorphic properties with explicit assessment of the suitability of a channel for certain types of aquatic organisms (Pennak 1971, Bovee and Cochnauer 1977, Binns and Eiserman 1979, Bisson *et al.* 1982, Beschta and Platts 1986, Sullivan *et al.* 1987, Hawkins *et al.* 1993, Stanford *et al.* 2005).

There are several reasons why stream ecologists classify and measure valley segments, stream reaches, and channel units. The first may simply be to describe physical changes in stream channels over time, whether in response to human impacts or to natural disturbances (Gordon *et al.* 1992, Buffington *et al.* 2003). A second reason for stream classification may be to group sampling areas into like physical units for purposes of comparison. This is often desirable when conducting stream surveys in different drainages. Classification of reach types and channel units enables investigators to extrapolate results to other areas with similar features (Hankin and Reeves 1988, Dolloff *et al.* 1993). A third objective for classification may be to determine the suitability of a stream for some type of deliberate channel alteration. Habitat restoration in streams and rivers with histories of environmental degradation is currently being undertaken in many locations, and some restoration procedures may be inappropriate for certain types of stream channels (National Research Council 1992, Pess *et al.* 2003). Successful rehabilitation requires that approaches be consistent with the natural hydraulic and geomorphic conditions of different reach types (Gordon *et al.* 1992, Buffington *et al.* 2003) and do not impede disturbance and recovery cycles (Reice 1994, Reeves *et al.* 1995). Finally, accurate description of stream reaches and channel units often is an important first step in describing the microhabitat requirements of aquatic organisms during their life histories or in studying the ecological processes that influence their distribution and abundance (Hynes 1970, Schlosser 1987, Weins 2002).

Geomorphically based stream reach and channel unit classification schemes continue to undergo refinement. Stream ecologists will do well to heed the advice of Balon (1982), who cautioned that nomenclature itself is less important than detailed descriptions of the meanings given to terms. Thus, it is important for investigators to be as precise as possible when describing what is meant by the terms of the classification scheme they have chosen. Although a number of stream reach and channel unit classification systems have been put forward, none has yet been universally accepted. In this chapter we focus on two classification schemes that can provide stream ecologists with useful tools for characterizing aquatic habitat at intermediate landscape scales: the Montgomery and Buffington (1997) model for valley segments and stream reaches, and the Hawkins *et al.* (1993) model for channel ("habitat") units. Both systems are based on hierarchies of topographic and fluvial characteristics, and both employ descriptors that are measurable and ecologically relevant. The Montgomery and Buffington (1997) classification provides a geomorphic, processed-oriented method of identifying valley segments and stream reaches, while the Hawkins *et al.* (1993) classification deals with identification and measurement of different types of channel units within a given reach. The methods described herein begin with a laboratory examination of maps

and photographs for preliminary identification of valley segments and stream reaches, and conclude with a field survey of channel units in one or more reach types.

A. Valley Segment Classification

Hillslopes and valleys are the principal topographic subdivisions of watersheds. Valleys are areas of the landscape where water converges and where eroded material accumulates. Valley segments are distinctive sections of the valley network that possess geomorphic properties and hydrological transport characteristics that distinguish them from adjacent segments. Montgomery and Buffington (1997) identified three terrestrial valley segment types: *colluvial, alluvial*, and *bedrock* (Figure 2.1). Colluvial valleys were subdivided into those with and without recognizable stream channels.

Valley segment classification describes valley form based on dominant sediment inputs and transport processes. The term *sediment* here includes both large and small inorganic particles eroded from hillslopes. Valleys can be filled primarily with *colluvium* (sediment and organic matter delivered to the valley floor by mass wasting [landslides] from adjacent hillslopes), which is usually immobile except during rare hydrologic events, or *alluvium* (sediment transported along the valley floor by streamflow), which may be frequently moved by the stream system. A third condition includes valleys that have little soil but instead are dominated by bedrock. Valley segments distinguish portions of the valley system in which sediment inputs and outputs are transport- or supply-limited (Figure 2.2). In transport-limited valley segments, the amount of sediment in the valley floor and its movements are controlled primarily by the frequency of high streamflows and debris flows (rapidly moving slurries of water, sediment, and organic debris) capable of mobilizing material in the streambed. In supply-limited valley segments, sediment movements are controlled primarily by the amount of sediment delivered to the segment by inflowing water. Valley segment classification does not allow forecasting of how the characteristics of the valley will change in response to altered discharge or sediment supply. Reach classification, according to Montgomery and Buffington (1997), is more useful for characterizing responses to such changes.

1. Colluvial Valleys

Colluvial valleys serve as temporary repositories for sediment and organic matter eroded from surrounding hillslopes. In colluvial valleys, fluvial (waterborne) transport
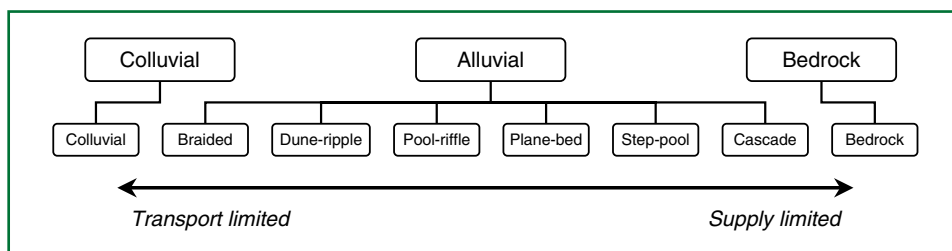
FIGURE 2.2 Arrangement of valley segment and stream reach types according to whether their substrates are limited by the supply of sediment from adjacent hillslopes or by the fluvial transport of sediment from upstream sources. After Montgomery and Buffington (1997).

is relatively ineffective at removing materials deposited on the valley floor. Consequently, sediment and organic matter gradually accumulates in headwater valleys until it is periodically flushed by debris flows in steep terrain, or excavated by periodic hydrologic expansion of the alluvial channel network in low-gradient landscapes. After removal of accumulated sediment by large disturbances, colluvial valleys begin refilling (Dietrich *et al.* 1986).

Unchanneled colluvial valleys are headwater valley segments lacking recognizable stream channels. They possess soils eroded from adjacent hillslopes, a property that distinguishes them from steep headwater valleys of exposed bedrock (Montgomery and Buffington 1997). The depth of colluvium in unchanneled colluvial valleys is related to the rate at which material is eroded from hillslopes and the time since the last valley excavating disturbance. The cyclic process of emptying and refilling occurs at different rates in different geoclimatic regions and depends on patterns of precipitation, geological conditions, and the nature of hillslope vegetation (Dietrich *et al.* 1986). Unchanneled colluvial valleys do not possess defined streams (Montgomery and Dietrich 1988), although seasonally flowing seeps and small springs may serve as temporary habitat for some aquatic organisms that are present in these areas.

Channeled colluvial valleys contain low-order streams immediately downslope from unchanneled colluvial valleys. Channeled colluvial valleys may form the uppermost segments of the valley network in landscapes of low relief, or they may occur where small tributaries cross floodplains of larger streams. Flow in colluvial channels tends to be shallow and ephemeral or intermittent. Because shear stresses (see Chapter 4) generated by streamflows are incapable of substantially moving and sorting deposited colluvium, channels in these valley segments tend to be characterized by a wide range of sediment and organic matter sizes. Episodic scour of channeled colluvial valleys by debris flows often governs the degree of channel incision in steep terrain, and like unchanneled colluvial valleys, cyclic patterns of sediment excavation periodically reset the depth of colluvium. Consequently, the frequency of sediment-mobilizing discharge or debris flows regulates the amount of sediment stored in colluvial valleys.

2. Alluvial Valleys

Alluvial valleys are supplied with sediment from upstream sources, and the streams within them are capable of moving and sorting the sediment at erratic intervals. The sediment transport capacity of an alluvial valley is insufficient to scour the valley floor to bedrock, resulting in an accumulation of valley fill primarily of fluvial origin. Alluvial valleys are the most common type of valley segment in many landscapes and usually contain streams of greatest interest to aquatic ecologists. They range from *confined*, a condition in which the hillslopes narrowly constrain the valley floor with little or no floodplain development, to *unconfined*, with a well-developed floodplain. A variety of stream reach types may be associated with alluvial valleys, depending on the degree of confinement, gradient, local geology and sediment supply, and discharge regime (Figure 2.3).

3. Bedrock Valleys

Bedrock valleys have little valley fill material and usually possess confined channels lacking an alluvial bed. Montgomery and Buffington (1997) distinguish two types of bedrock valleys: those sufficiently steep to have a transport capacity greater than the
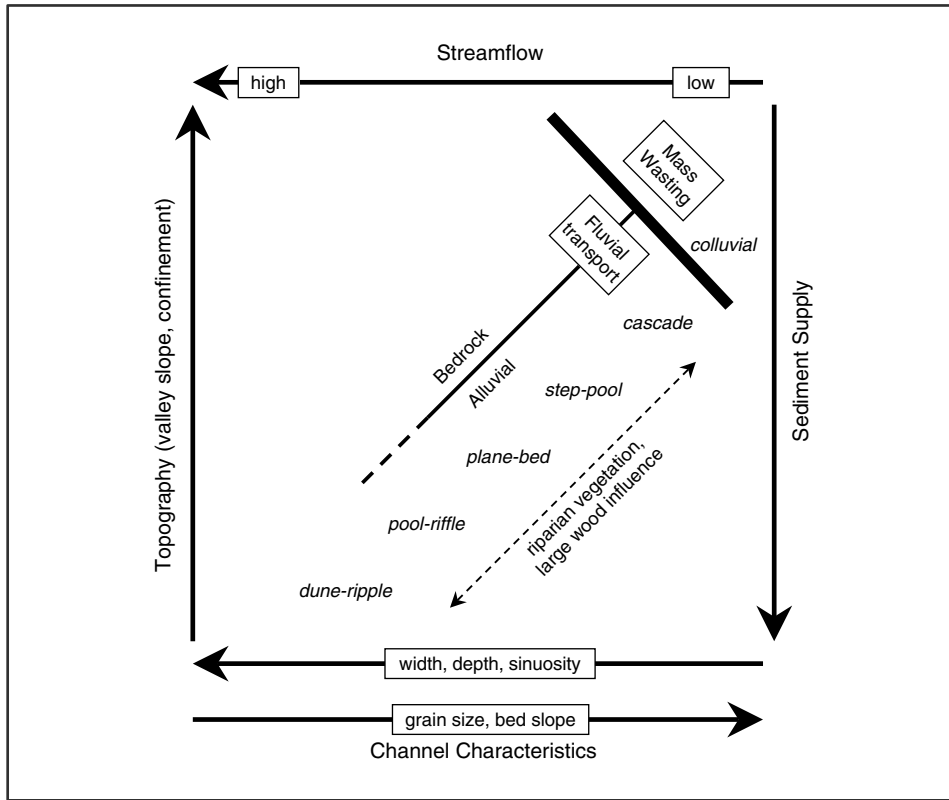
FIGURE 2.3 Influence of watershed conditions, sediment supply, and channel characteristics on reach morphology. After Buffington *et al.* (2003).

sediment supply and thereby remain permanently bedrock floored, and those associated with low-order streams recently excavated to bedrock by debris flows.

B. Channel Reach Classification

Channel reaches consist of repeating sequences of specific types of channel units (e.g., pool-riffle-bar sequences) and specific ranges of channel characteristics (slope, sediment size, width–depth ratio), which distinguish them in certain aspects from adjoining reaches (Table 2.2). Although reach types are associated with specific ranges of channel characteristics (slope, grain size, etc.) (Buffington *et al.* 2003), those values are not used for classification. Rather, reach types are identified in terms of channel morphology (shape) and observed processes. Transition zones between adjacent reaches may be gradual or sudden, and exact upstream and downstream reach boundaries may be a matter of some judgment. Colluvial valley segments can possess colluvial and bedrock reach types, and bedrock valleys can host bedrock and alluvial reach types (Table 2.2), but alluvial valleys typically exhibit varieties of alluvial reach types. Montgomery and Buffington (1997) state that reach boundaries in alluvial valleys are related to the supply and characteristics of sediment and to the power of the stream to mobilize its bed (Figure 2.3). Specifically, they

	Colluvial	**Bedrock**	**Cascade**	**Step-pool**	**Plane-bed**	**Pool-riffle**	**Dune-ripple**	**Braided**
TABLE 2.2	Characteristics of Different Types of Stream Reaches. Modified from Montgomery and Buffington (1997).							

	Colluvial	Bedrock	Cascade	Step-pool	Plane-bed	Pool-riffle	Dune-ripple	Braided
Predominant bed material	variable	bedrock	boulder	cobble/boulder	gravel/cobble	gravel	sand	variable (sand to boulder)
Bedform pattern	variable	variable	chaotic	vertically oscillatory	none	laterally oscillatory	multilayered	laterally oscillatory
Dominant roughness elements	banks, boulders, large wood	streambed, banks	boulders, banks	bedforms (steps, pools) boulders, large wood, banks	boulders and cobbles, banks	bedforms (bars, pools) boulders and cobbles, large wood, sinuosity, banks	sinuosity, bedforms (dunes, ripples, bars), banks, large wood	bedforms (bars, pools), boulders and cobbles
Dominant sediment sources	hillslope, debris flows	fluvial, hillslope, debris flows	fluvial, hillslope, debris flows	fluvial, hillslope, debris flows	fluvial, bank erosion, debris flows	fluvial, bank erosion, inactive channels, debris flows	fluvial, bank erosion, inactive channels	fluvial, bank erosion, debris flows, glaciers
Typical slope (%)	>20	variable	4–25	2–8	1–4	0.1–2	<0.1	<3
Typical confinement	strongly confined	strongly confined	strongly confined	moderately confined	variable	unconfined	unconfined	variable
Pool spacing (channel widths)	variable	variable	<1	1–4	none	5–7	5–7	variable
Bankfull recurrence interval (years)	variable	variable	variable	variable	1–2	1–2	1–2	variable

recognized six alluvial reach types, although they further recognized that intermediate reach types also occur.

1. Cascade Reaches

This reach type is characteristic of the steepest alluvial channels, with gradient typically ranging from 4 to 25%. A few small, turbulent pools may be present in cascade reaches, but the majority of flowing water tumbles over and around boulders and large wood. The boulders are supplied from adjacent hillslopes or from periodic debris-flow deposition. Waterfalls ("hydraulic jumps") of various sizes are abundant in cascade reaches. The large size of particles relative to water depth effectively prevents substrata mobilization during typical flows. Although cascade reaches may experience debris flows, sediment movement is predominantly fluvial. The cascading nature of water movement in this reach type is usually sufficient to remove all but the largest particles of sediment (cobbles and boulders) and organic matter. What little fine sediment and organic matter occurs in cascade reaches remains trapped behind boulders and logs, or it is stored in a few pockets where reduced velocity and turbulence permit deposition. The rapid flushing of fine sediment from cascade reaches during moderate to high flows suggests that transport from this reach type is limited by the supply of sediment recruited from upstream sources (Figure 2.2).

2. Step-pool Reaches

Step-pool reaches, with typical gradients of 2–8%, possess discrete channel-spanning accumulations of boulders and logs that form a series of steps alternating with pools containing finer substrata. Step-pool reaches tend to be straight and have high gradients, coarse substrata (cobbles and boulders), and small width to depth ratios. Pools and alternating bands of channel-spanning flow obstructions typically occur at a spacing of every 1–4 channel widths in step-pool reaches, although step spacing increases with decreasing channel slope (Grant *et al.* 1990). A low supply of sediment, steep gradient, infrequent flows capable of mobilizing coarse streambed material, and heterogeneous sediment composition appear to favor the development of this reach type.

The capacity of step-pool reaches to temporarily store fine sediment and organic matter generally exceeds the sediment storage capacity of cascade reaches. Flow thresholds necessary to transport sediment and mobilize channel substrata are complex in step-pool reaches. Large bed-forming structures (boulders and large wood) are relatively stable and move only during extreme flows. In very high streamflows the channel may lose its stepped profile, but step-pool morphology becomes reestablished during the falling limb of the hydrograph (see Chapter 3, Whittaker 1987). During high flows, fine sediment and organic matter in pools is transported over the large, stable bed-forming steps.

3. Plane-bed Reaches

Plane-bed stream reaches, with gradients typically 1–4%, lack a stepped longitudinal profile and instead are characterized by long, relatively straight channels of uniform depth. They are usually intermediate in gradient and relative submergence (the ratio of bankfull flow depth to median particle size) between steep, boulder dominated cascade and step-pool reaches, and the more shallow gradient pool-riffle reaches. At low to moderate flows, plane-bed stream reaches may possess large boulders extending above

the water surface, forming midchannel eddies. However, the absence of channel-spanning structures or significant constrictions by streambanks inhibits pool development. Particles in the surface layer of plane-bed reaches typically are larger than those in subsurface layers and form an armor layer over underlying finer materials (Montgomery and Buffington 1997). This armor layer prevents transport of fine sediments except during periods when flow is sufficient to mobilize armoring particles.

4. Pool-riffle Reaches

This reach type is most commonly associated with small to midsized streams and is a very prevalent type of reach in alluvial valleys of low to moderate gradient (1–2%). Pool-riffle reaches tend to possess lower gradients than the three previous reach types and are characterized by an undulating streambed that forms riffles and pools associated with gravel bars. Also, unlike most cascade, step-pool, and plane-bed reaches, the channel shape of pool-riffle reaches is often sinuous and contains a predictable and often regular sequence of pools, riffles, and bars in the channel. Pools are topographic depressions in the stream bottom and bars form the high points of the channel. Riffles are located at crossover areas from pools to bars. At low streamflow, the water meanders around bars and through pools and riffles that alternate from one side of the river to the other. Pool-riffle reaches form naturally in alluvial channels of fine to moderate substrata coarseness (Leopold *et al.* 1964, Yang 1971) with single pool-riffle-bar sequences found every 5–7 channel widths (Keller and Melhorn 1978). Large wood, if present, anchors the location of pools and creates upstream sediment terraces that form riffles and bars (Lisle 1986, Bisson *et al.* 1987). Streams rich in large wood tend to have erratic and complex channel morphologies (Bryant 1980, Montgomery *et al.* 2003).

Channel substrata in pool-riffle reaches are mobilized annually during freshets. At bankfull flows, pools and riffles are inundated to such an extent that the channel appears to have a uniform gradient, but local pool-riffle-bar features emerge as flows recede. Movement of bed materials at bankfull flow is sporadic and discontinuous (Montgomery and Buffington 1997). As portions of the surface armor layer are mobilized, finer sediment underneath is flushed, creating pulses of scour and deposition. This process contributes to the patchy nature of pool-riffle reaches, whose streambeds are among the most spatially heterogeneous of all reach types (Buffington and Montgomery 1999).

5. Dune-ripple Reaches

Dune-ripple stream reaches consist of low gradient (<1%), meandering channels with predominantly sand substrata. This reach type generally occurs in higher order channels within unconstrained valley segments and exhibits less turbulence than reach types with high gradients. Shallow and deep water areas are present and point bars may be present at meander bends. As current velocity increases over the fine-grained substrata of dune-ripple reaches, the streambed is molded into a predictable succession of bedforms, from small ripples to a series of large dunelike elevations and depressions. Sediment movement occurs at all flows and is strongly correlated with discharge. A well-developed floodplain typically is present. The low gradient, continuous transport of sediment, and presence of ripples and dunes distinguish this reach type from pool-riffle reaches.

6. Braided Reaches

Braided reaches possess multithread channels with low to moderate gradients (<3%) and are characterized by large width–depth ratios and numerous bars scattered throughout the channel (Buffington *et al.* 2003). Individual braid threads typically have a pool-riffle morphology, with pools commonly formed at the confluence of two braids. Bed material varies from sand to cobble and boulder, depending on channel gradient and local sediment supply. Braiding results from high sediment loads or channel widening caused by destabilized banks. Braided channels commonly occur in glacial outwash zones and other locations overwhelmed by high sediment supply (e.g., downstream of massive landslides or volcanic eruptions) or in places with weak, erodible banks (e.g., river corridors that have lost vegetative root strength because of riparian cattle grazing or riparian clear cutting or in semiarid regions where riparian vegetation is naturally sparse) (Buffington *et al.* 2003). In braided reaches the location of bars change frequently, and the channel containing the main flow can often move laterally over short periods of time.

7. Forced Reaches

Flow obstructions such as large wood debris and bedrock projections can locally force a reach morphology that would not otherwise occur (Montgomery and Buffington 1997). For example, wood debris introduced to a plane-bed channel may create local pool scour and bar deposition that forces a pool-riffle morphology (Table 2.1). Similarly, wood in cascade or bedrock channels may dam upstream sediment and create downstream plunge pools, forming a step-pool morphology. The effects of wood debris on streamflow, sediment transport, and pool formation are further discussed by Buffington *et al.* (2002).

C. Channel Unit Classification

Channel units are relatively homogeneous localized areas of the channel that differ in depth, velocity, and substrata characteristics from adjoining areas. The most generally used channel unit terms for small to midsize streams are riffles and pools. Individual channel units are created by interactions between flow and roughness elements of the streambed. Definitions of channel units usually apply to conditions at low discharge. At high discharge, channel units are often indistinguishable from one another, and their hydraulic properties differ greatly from those at low flows.

Different types of channel units in close proximity to one another provide organisms with a choice of habitat, particularly in small streams possessing considerable physical heterogeneity (Hawkins *et al.* 1993). Channel unit classification is therefore quite useful for developing an understanding of the distribution and abundance of aquatic plants and animals in patchy stream environments. Channel units are known to influence nutrient exchanges (Triska *et al.* 1989, Aumen *et al.* 1990), algal abundance (Tett *et al.* 1978, Murphy 1998), production of benthic invertebrates (Huryn and Wallace 1987), invertebrate diversity (Hawkins 1984), and the distribution of fishes (Angermeier 1987, Bisson *et al.* 1988, Schlosser 1991). The frequency and location of different types of channel units within a reach can be affected by a variety of disturbances, including anthropogenic disturbances that remove structural roughness elements such as large wood (Lisle 1986, Sullivan *et al.* 1987, Woodsmith and Buffington 1996, Elosegi and Johnson 2003) or impede the ability of a stream to interact naturally with its adjacent riparian zone (Beschta and Platts 1986, Pinay *et al.* 1990). Channel unit classification is a useful tool for
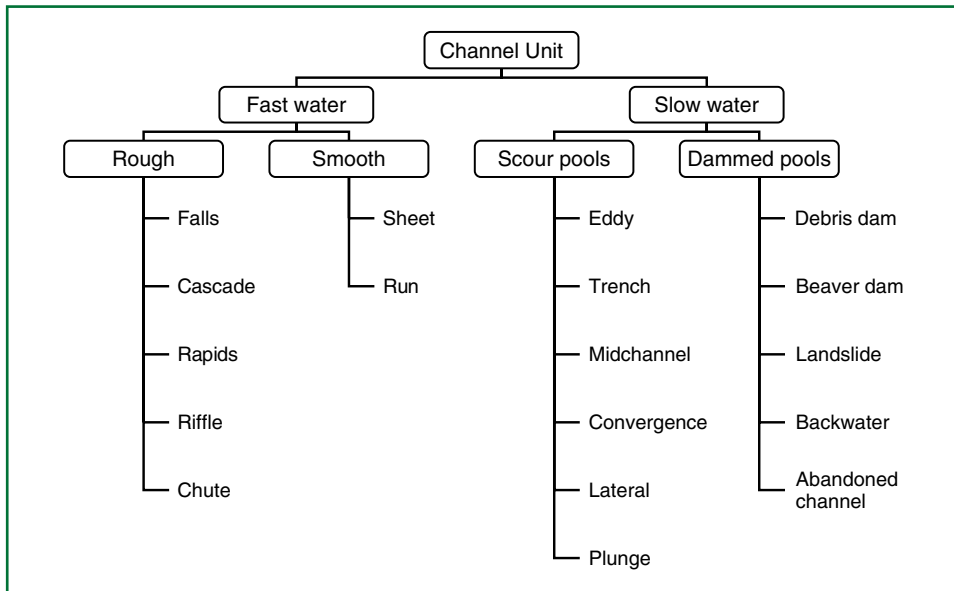
FIGURE 2.4 Hierarchical subdivision of channel units in streams. After Hawkins *et al.* (1993).

understanding the relationships between anthropogenically induced habitat alterations and aquatic organisms.

Hawkins *et al.* (1993) modified an earlier channel unit classification system (Bisson *et al.* 1982) and proposed a three-tiered system of classification (Figure 2.4) in which investigators could select the level of habitat resolution appropriate to the question being addressed. The first level was subdivided into fast water ("*riffle*") from slow water ("*pool*") units. The second level distinguished fast water units having rough ("turbulent") versus smooth ("nonturbulent") water surfaces, and slow water units formed by scour from slow water units formed by dams. Strictly speaking, all river flows are turbulent according to hydraulic principles. Consequently, we use the terms "rough" and "smooth" rather than the "turbulent" and "nonturbulent" terms proposed by Hawkins *et al.* (1993). The third level of classification further subdivided each type of fast and slow water unit based on characteristic hydraulic properties and the principal kind of habitat-forming structure or process.

1. Rough Fast Water Units

The term "fast water" is a relative term that describes current velocities observed at low to moderate flows and is meant only to distinguish this class of channel unit from other units in the same stream with "slow water." Most of the time, but not always, slow water units will be deeper than fast water units at a given discharge. The generic terms *riffle* and *pool* are frequently applied to fast and slow water channel units, respectively, although these terms convey limited information about geomorphic or hydraulic characteristics of a stream. Current velocity and depth are the main criteria for separating riffles from pools in low- to midorder stream channels. Although there are no absolute values of velocity or depth that identify riffles and pools, they are by definition separated by depth.

TABLE 2.3	Types of Rough and Smooth Fast Water Channel Units and the Relative Rankings of Variables Used to Distinguish Them. Rankings are in descending order of magnitude where a rank of 1 denotes the highest value of a particular parameter. Step development is ranked by the abundance and size of hydraulic jumps within a channel unit. From Hawkins *et al.* (1993).

	Gradient	Supercritical Flow	Bed Roughness	Mean Velocity	Step Development
Rough					
Falls	1	n/a	n/a	1	1
Cascade	2	1	1	2	2
Chute	3	2	4	3	5
Rapids	4	3	2	4	3
Riffle	5	4	3	5	4
Smooth					
Sheet	variable	6	6	6	5
Run	6	5	5	7	5

Pools are not shallow and riffles are not deep. However, pools can contain fast or slow waters, while riffles are only fast.

Hawkins *et al.* (1993) recognized five types of rough fast water channel units (Table 2.3). Channel units are classified as rough as Froude number increases (see Chapter 4). Hydraulic jumps, sufficient to entrain air bubbles and create localized patches of white water, approach and can exceed critical flow. In contrast, the appearance of the flow is much more uniform in smooth fast water units. Rough fast water channel units are listed in Table 2.3 in approximate descending order of gradient, bed roughness, current velocity, and abundance of hydraulic steps.

Falls are essentially vertical drops of water and are commonly found in bedrock, cascade, and step-pool stream reaches. *Cascade* channel units consist of a highly turbulent series of short falls and small scour basins, frequently characterized by very large sediment sizes and a stepped longitudinal profile. They are prominent features of bedrock and cascade reaches. *Chute* channel units are typically narrow, steep slots in bedrock. They are common in bedrock reaches and also occur in cascade and step-pool reaches. *Rapids* are moderately steep channel units with coarse substrata, but unlike cascades possess a somewhat planar (vs. stepped) longitudinal profile. Rapids are the dominant fast water channel unit of plane-bed stream reaches. *Riffles* are the most common type of rough fast water in low gradient (<3%) alluvial channels and may be found in plane-bed, pool-riffle, dune-ripple, and braided reaches. The particle size of riffles tends to be somewhat finer than that of the other rough fast water units, since riffles are shallower than rapids and generally have lower tractive force to mobilize the stream bed (see Chapter 4).

2. Smooth Fast Water Units

Hawkins *et al.* (1993) recognized two types of smooth fast water units. *Sheet* channel units are rare in many watersheds but may be common in valley segments dominated by bedrock. Sheets occur where shallow water flows uniformly over smooth bedrock of variable gradient; they may be found in bedrock, cascade, or step-pool reaches, but they are generally highly isolated as true sheet flow is highly rare in stream systems. *Run*

channel units are fast water units of shallow gradient, typically with substrata ranging in size from sand to cobbles. They are characteristically deeper than riffles and because of their smaller substrata have little if any supercritical flow, giving them a smooth appearance. Runs are common in pool-riffle, dune-ripple, and braided stream reaches, usually in mid- and higher-order channels.

3. Scour Pools

There are two general classes of slow water channel units: pools created by scour that forms a depression in the streambed and pools created by the impoundment of water upstream from an obstruction to flow (Table 2.4). Scour pools can be created when discharge is sufficient to mobilize the substrata at a particular site, while dammed pools can be formed under any flow condition. Hawkins *et al.* (1993) recognized six types of scour pools.

Eddy pools are the result of large flow obstructions along the edge of the stream or river. Eddy pools are located on the downstream side of the structure and are usually proportional to the size of the obstruction. Eddy pools are often associated with large wood deposits or rock outcrops and boulders and can be found in virtually all reach types.

Trench pools, like chutes, are usually located in tightly constrained, bedrock dominated reaches. They are characteristically U-shaped in cross-sectional profile and possess highly resistant, nearly vertical banks. Trench pools can be among the deepest of the slow water channel units created by scour, and their depth tends to be rather uniform throughout much of their length, unlike other scour pool types. Although often deep, trench pools may possess relatively high current velocities.

Midchannel pools are formed by flow constrictions that focus scour along the main axis of flow in the middle of the stream. Midchannel pools are deepest near the head. This type of slow water channel unit is very common in cascade, step-pool, and pool-riffle reaches. Flow constriction may be caused by laterally confined, hardened banks (bridge abutments are good examples) or by large flow obstructions such as boulders or woody debris, but an essential feature of midchannel pools is that the direction of water movement around an obstruction is not diverted toward an opposite bank.

Convergence pools result from the confluence of two streams of somewhat similar size. In many respects convergence pools resemble midchannel pools except that there are two main water entry points, which may result in a pattern of substrata particle sorting in which fines are deposited near the head of the pool in the space between the two inflowing channels. Convergence pools can occur in any type of alluvial stream reach.

Lateral scour pools occur where the channel encounters a resistant streambank or other flow obstruction near the edge of the stream. Typical obstructions include bedrock outcrops, boulders, large wood, or gravel bars. Many lateral scour pools form next to or under large, relatively immovable structures such as accumulations of logs or along a streambank that has been armored with rip-rap or other material that resists lateral channel migration. Water is deepest adjacent to the streambank containing the flow obstruction and shallowest next to the opposite bank. Lateral scour pools are very common in step-pool, pool-riffle, dune-ripple, and braided reaches. In pool-riffle and dune-ripple reaches, lateral scour pools form naturally at meander bends in gravel-bed streams even without large roughness elements (Leopold *et al.* 1964, Yang 1971).

Plunge pools result from the vertical fall of water over a full spanning obstruction onto the streambed. The full spanning obstruction creating the plunge pool is located at the head of the pool, and the waterfall can range in height from less than a meter to

	Location	Longitudinal Profile	Cross-sectional Profile	Substrate Features	Forming Constraint
TABLE 2.4					Characteristics of Slow Water Channel Units. Location denotes whether the unit is likely to be associated with the thalweg of the channel (the main part of the flow) or adjacent to a bank. Longitudinal and cross-sectional profiles refer to the deepest point in the unit relative to the head, middle, or tail region of the unit. Substrata characteristics refer to the extent of particle sorting (i.e., particle uniformity) and resistance to scour. The channel unit forming constraint describes the feature most likely to cause pooling. Modified from Hawkins *et al.* (1993).

	Location	Longitudinal Profile	Cross-sectional Profile	Substrate Features	Forming Constraint
Scour pools					
Eddy	bank	middle	middle	surface fines, not resistant to scour	flow obstruction causing lateral deflection
Trench	thalweg	uniform	uniform	bedrock or sorted, resistant to scour	bilateral resistance
Midchannel	thalweg	middle	middle	sorted, variable resistance to scour	constriction at upstream end
Convergence	thalweg	middle	middle	sorted, variable resistance to scour	convergence of two channels
Lateral	thalweg	head or middle	side	sorted, variable resistance to scour	flow obstruction causing lateral deflection
Plunge	thalweg	head	upstream or middle	sorted, variable resistance to scour	full-spanning obstruction causing waterfall
Dammed pools					
Debris dam	thalweg	tail	highly variable	usually sorted, not resistant to scour	large woody debris dam of fluvial origin
Beaver dam	thalweg	tail	highly variable	surface fines, not resistant to scour	beaver dam
Landslide dam	thalweg	tail	highly variable	often unsorted, variable resistance to scour	organic and inorganic matter delivered by mass wasting from adjacent hillslope
Backwater	bank	tail	highly variable	unsorted with surface fines, not resistant to scour	obstruction at tail impounding water along margin of main channel
Abandoned channel	floodplain	highly variable	highly variable	unsorted with surface fines, not resistant to scour	lateral meander bars that isolate an overflow channel from the main channel

hundreds of meters, as long as the force of the fall is sufficient to scour the bed. A second, far less common type of plunge pool occurs in higher-order channels where the stream passes over a sharp geological discontinuity such as the edge of a plateau, forming a large falls with a deep pool at the base. Depending on the height of the waterfall and the composition of the substrata, plunge pools can be quite deep. Overall, plunge pools are most abundant in small, steep headwater streams, especially those with bedrock, cascade, and step-pool reaches.

4. Dammed Pools

Dammed pools are created by the impoundment of water upstream from a flow obstruction, rather than by scour downstream from the obstruction. They are distinguished by the type of material causing the water impoundment and by their location in relation to the thalweg (Table 2.4). The rate at which sediment fills dammed pools depends on sediment generation from source areas and fluvial transport from upstream reaches. Due to their characteristically low current velocities, dammed pools often have more surface fines than scour pools and fill with sediment at a much more rapid rate. However, some types of dammed pools tend to possess more structure and cover for aquatic organisms than scour pools because of the complex arrangement of material forming the dam. Additionally, dammed pools can be very large, varying with the height of the dam and the extent to which it blocks the flow. Highly porous dams result in little impoundment. Well-sealed dams usually fill to the crest of the dam, creating a spill.

Hawkins *et al.* (1993) identified five types of dammed pools, three of which occur in the main channel of streams. *Debris* dam pools are typically formed at the terminus of a debris flow or where large pieces of wood float downstream at high discharge and lodge against a channel constriction. The characteristic structure of debris dams consists of one or a few large key pieces that hold the dam in place and that trap smaller pieces of wood and sediment that comprise the matrix.

Beaver dam pools, the only channel unit of natural biogenic origin, are unlike debris dam pools in that they usually lack very large key pieces but consist instead of tightly woven smaller pieces sealed on the upstream surface with fine sediment. Some beaver dams may exceed two meters in height, but most dams in stream systems are about a meter or less high. In watersheds with high seasonal runoff, beaver dams may breach and be rebuilt annually. In such instances, fine sediments stored above the dam are flushed when the dam breaks.

Landslide dam pools form when a landslide from an adjacent hillslope blocks a stream, causing an impoundment. Dam material consists of a mixture of coarse and fine sediment and, in forested terrain, woody debris. When landslides occur, some or most of the fine sediment in the landslide deposit may be rapidly transported downstream, leaving behind structures too large to be moved by the flow. Main channel landslide pools are located primarily in laterally constrained reaches of relatively small streams. They are most abundant in confined reaches (step-pool and cascade reaches) where hillslopes are directly coupled to the channel, although some are found in moderately confined pool-riffle and plane-bed reaches of larger-order streams. Dammed pools are nearly always less abundant than scour pools in alluvial channels, due to the rapidity with which they fill with sediment and the temporary nature of most dams.

Two types of dammed pools located away from the main channel are found primarily at low flows. *Backwater* pools occur along the bank of the main stream at an downstream

end of an upstream disconnected floodplain channel. Backwater pools often appear as a diverticulum from the main stream and possess water flowing slowly in an eddy pattern. Pool-riffle, dune-ripple, and braided reaches are most likely to possess this type of channel unit.

Abandoned channel pools have no surface water connections to the main channel. They are formed by bar deposits in secondary channels that are isolated at low flow. Abandoned channel pools are floodplain features of pool-riffle, dune ripple, and braided reaches that may be ephemeral or maintained by subsurface flow (see Chapter 6).

II. GENERAL DESIGN

A. Site Selection

It is generally impossible to locate examples of every type of valley segment, stream reach, and channel unit in one watershed due to regional differences in geology and hydrologic regimes. Instead, it is likely that potential study sites will consist of certain commonly occurring local reach types. In the laboratory, maps and photographs will be used to determine approximate reach boundaries based on stream gradients, degree of valley confinement, channel meander patterns, or significant changes in predominant rock type. The main goal of the laboratory portion of this chapter is to practice map skills and to locate two or more distinctive stream reach types.

B. General Procedures

While it is possible to infer valley segment and reach types from maps and photographs, preliminary classification should be verified by a visit to the sites. Identification of channel units from low elevation aerial photographs, especially for small streams enclosed within a forest canopy, is virtually impossible and always requires a field survey. In the laboratory, the stream of interest can be divided into sections based on average gradient and apparent degree of valley confinement (Montgomery and Buffington, 1998). Topographic changes in slope can provide important information regarding reach boundaries (Baxter and Hauer 2000). The scale of topographic maps (including USGS 7.5 minute series maps) may or may not allow identification of key changes in stream gradient and valley confinement that mark reach transitions in very small streams. Maps may or may not provide accurate information on the sinuosity of the stream or the extent of channel braiding, depending on the size of the stream and reach you are studying and the age and resolution of the map or image you are working with. Nonetheless, topographic maps are essential for plotting changes in the elevational profile of a stream, as well as changes in valley confinement.

Aerial photographs are often available from natural resource management agencies and should be used to supplement information extracted from maps. Aerial photographs can be used to accurately locate changes in channel shape in streams not obscured by forest canopies. Orthographic photographs provide a three-dimensional, if somewhat exaggerated, perspective of landscape relief but require stereoscopic map reading equipment that optically superimposes offset photos. This equipment can range from pocket stereoscopes costing $20 to mirror reflecting stereoscopes costing over $2,000. Low-altitude aerial photographs (1:12,000 scale or larger) are most useful and should be examined whenever available. Geological and soils maps of the area will help identify

boundaries between geological formations, another important clue to the location of different reach types. Vegetative maps or climatological maps (e.g., rainfall or runoff), if available, provide additional information about the setting of the stream. Landsat imagery can be helpful at large landscape scales but does not provide the resolution needed for designation of reach boundaries in small streams. Shaded relief images made from laser altimetry, or LiDAR (Light Detection and Ranging), data provide highly detailed views of topographic relief and can help establish reach transitions and are useful for understanding channel migration history (National Center for Airborne Laser Mapping 2005).

Once the stream has been subdivided into provisional reach boundaries in the laboratory, contrasting sites are visited and all or part of the reach(es) of interest is surveyed on foot using the criteria in Tables 2.3 and 2.4 to identify channel units. This is often a time-consuming process, depending on the accessibility of the reach, its length and riparian characteristics, and the time required to conduct an inventory of channel units within the reach. Surveys of channel units in small to midsize streams typically involve teams of two to three people covering 1–5 km day^{-1}. Representative sections of a reach can be studied, provided the sections include examples of each type of channel unit present in the reach as a whole (Dolloff *et al.* 1993). A useful rule of thumb is that reach subsamples should be at least 30–50 channel widths long; for example, a survey of channel units in a reach with an average channel width of 10 m should be at least 300–500 m long. During the survey the team should verify that the preliminary classification of valley segment and reach type in the laboratory was correct. Any significant changes in reach character should be noted, particularly if the stream changes from one reach type to another. The valley segment types most often surveyed by stream ecologists will be alluvial and bedrock (colluvial reaches also are easily recognized). Diagnostic reach characteristics are given in Table 2.2.

Surveys of channel unit composition can be used simply to determine the presence and number of each type of unit in the reach. More often, however, investigators wish to establish the percent of total wetted area or volume in each channel unit type on the date the stream was surveyed. Simple counts of the number and type of channel unit can be completed almost as fast as it takes to walk the reach, but estimates of surface area or volume can require considerable time, depending on the complexity of the channel and size of the units. Highly accurate estimates of area and volume involve many length, width, and depth measurements of each unit, increasingly measured in large channels with precise Global Positioning System (GPS) surveying equipment. Visual estimation of the surface area of individual channel units has proven to be a reasonably accurate and much less time-consuming technique (Hankin and Reeves 1988, Dolloff *et al.* 1993). However, visual estimates must be periodically calibrated by comparing them with careful measurements of the same channel units. Part of this exercise will involve performing such a comparison.

In conducting channel unit surveys the question inevitably arises: "What is the relative size of the smallest possible unit to be counted?" For channels with complex topographic features and considerable hydraulic complexity, this is a challenging question. Fast water units possess some areas of low current velocity, and slow water units usually have swiftly flowing water in them at some point. Location of channel unit boundaries for survey purposes is almost always subjective. Except for waterfalls, transitions from one unit to the next are gradual. In general, an area should be counted as a separate unit if (1) its overall physical characteristics are clearly different from those of adjacent units, and (2) its size is significant relative to the size of the wetted channel. A guideline for what

constitutes "significant" is that the greatest dimension of the channel unit should equal or exceed the average wetted width of the reach for units in the stream's thalweg and one-half the average wetted width of the reach for units along the stream's margin. It is quite possible (and should be expected) that channel units will not all be arranged in linear fashion along the reach but that some units will be located next to each other, depending on the presence of flow obstructions and channel braiding.

Channel unit surveys challenge investigators to balance the accuracy of characterizing stream conditions over an entire reach against the precision obtained by carefully mapping a limited subsection of the reach (Poole *et al.* 1997). The greater the desired precision, the more time will be required for the survey and the less the area that can be covered within a given time. Rapid techniques for visually estimating channel unit composition in stream reaches exist (Hankin and Reeves 1988) as well as precise survey methods for mapping the fine details of channel structure at a scale of one to several units (Gordon *et al.* 1992). What technique is appropriate will be governed by the nature of the research topic. In all cases, investigators must keep in mind that variations in discharge can strongly influence the relative abundance of different channel unit types; therefore, it is often desirable to repeat the survey at a variety of flows.

Although inventories of channel units in reaches of small streams can be conducted by one person, it is much easier and safer for surveys to be carried out by teams of at least two to three people. Because it is necessary to measure lengths and widths repeatedly, each crew member can be assigned a different task. Although practiced survey crews become proficient at identifying channel unit boundaries and maximizing data gathering efficiency, it is important to work slowly and deliberately. It is far better to take the time to collect accurate data than to be in a hurry to complete the reach survey; further, the risk of accidents declines with careful planning and time management and cautious attention to detail. Work safely.

III. SPECIFIC METHODS

A. Basic Method 1: Stream Reach Classification

1. Laboratory Protocols

1. Select a watershed. Assemble topographic maps, aerial photographs, and other information pertinent to the area. Within the watershed, select a stream or streams of interest.
2. With the aid of the topographic map, construct a longitudinal profile of the channel beginning at the mouth of the stream and working toward the headwaters. Use a map wheel (also called a curvimeter or map measure) or a planimeter to measure distance along the blue line that marks the stream. If a map wheel or planimeter is not available, a finely graduated ruler may be substituted. In either case, be sure to calibrate the graduations on the map wheel, planimeter, or ruler against the map scale. Record the elevation and distance from the mouth each time a contour line intersects the channel. Plot the longitudinal profile of the stream with the stream source nearest the vertical axis (Figure 2.5). If Geographic Information System (GIS) coverage of the area is available, use the appropriate data queries to determine channel length and longitudinal profile.
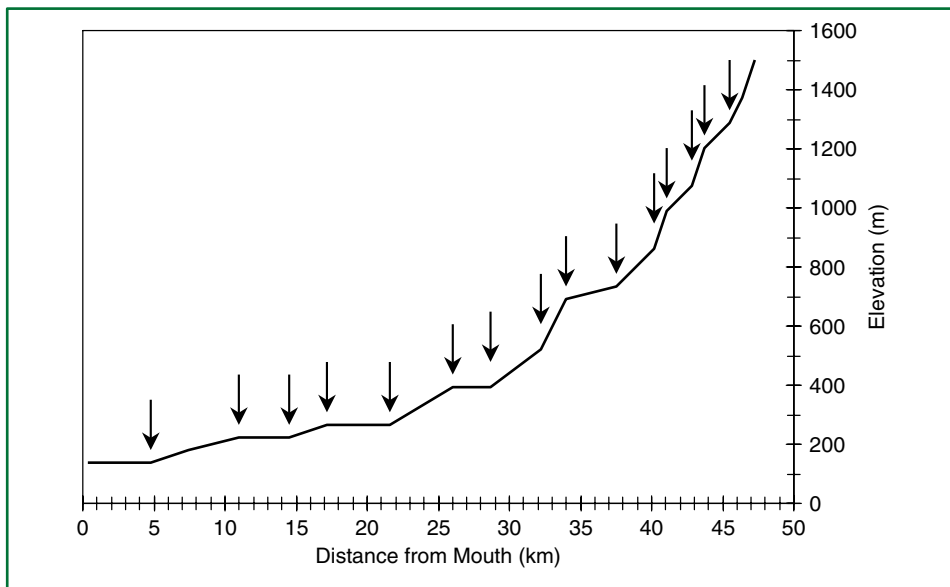
FIGURE 2.5 Hypothetical example of a stream profile constructed from a topographic map. Arrows denote changes in gradient that may mark reach boundaries.

3. Visually locate inflection points on the stream profile (Figure 2.5). These points often mark important reach transitions. Compute the average channel slope in each segment according to the following formula:

$$S = \frac{E_u - E_d}{L} \tag{2.1}$$

where S = average slope, E_u = elevation at upstream end of stream reach, E_d = elevation at downstream end of stream reach, and L = reach length. Remember to use common distance units for both numerator and denominator.

4. Examine the shape of the contour lines intersecting the stream to determine the approximate level of valley confinement in each segment. The width of the channel will not be depicted on most topographic maps, but the general shape and width of the valley floor will indicate valley confinement (Figure 2.6).

5. With the aid of a stereoscopic map reader, magnifying lens, or dissecting microscope, examine photographs of the stream segments identified on the topographic map. If it is possible to see the exposed (unvegetated) channel in the photographs, estimate the width of the exposed channel and compare it to the estimated width of the flat valley floor. Use the following guidelines to determine the approximate degree of confinement for the reach:

Valley Floor Width <2 Channel Widths	Strongly Confined
Valley Floor Width = 2–4 Channel Widths	Moderately Confined
Valley Floor Width >4 Channel Widths	Unconfined
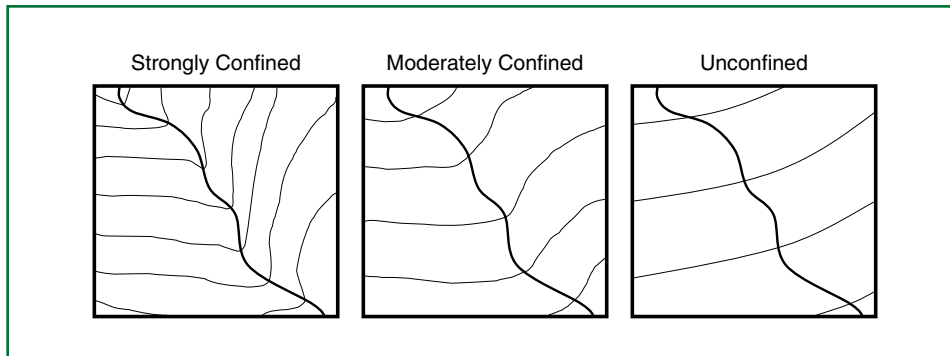
FIGURE 2.6 Appearance of strongly confined, moderately confined, and unconfined channels on topographic maps.

6. Compare average gradients and valley floor widths of each segment on the longitudinal stream profile with geological, soils, vegetation, and/or climatological maps of the watershed (as available). Changes in the boundaries shown on these maps may help in more precisely locating reach boundaries and in forming hypotheses about reach conditions that can be evaluated during visits to the sites. From all available evidence, determine the most likely valley segment and reach type (or range of types) for each segment based on the features summarized in Table 2.2. Select one or more reaches for site surveys.

2. Field Protocols

It may be possible to combine certain aspects of the field survey in this exercise with field methods discussed elsewhere in this book. One reach may be surveyed on one field trip and a second reach surveyed on a different field trip.

1. Upon arrival at the site, inspect the stream channel, adjacent valley floor, and hillslopes to verify the accuracy of preliminary valley segment and reach classification. If it is possible to do so (for example, from a vantage point that permits a panoramic view of the valley floor), locate landmarks that mark reach boundaries and that are easily visible from the stream itself.
2. If the reach is too long to complete the exercise within two to four hours (e.g., >500 m), select a representative section of the reach for the channel unit survey. Location of representative sections may be based on ease of access, but the section should typify the reach as a whole and be long enough to likely contain all types of channel units in the reach (30–50 channel widths). Use the descriptions of channel unit types in Tables 2.3 and 2.4 to identify the units. If reference photographs of different types of channel units are available, refer to them when necessary.
3. If optical or laser rangefinders will be used to measure distances (recommended for all but the smallest streams), calibrate them at the beginning of each field trip them by measuring the distance between two points with a tape and adjusting the readings on the rangefinders to match the known distance. Optical rangefinders, in particular, can become misaligned if dropped and should be recalibrated frequently.

4. If surface area will be estimated visually, it may be helpful to calibrate the "eye" of the observer by placing several rectangles or circles of plastic of known area on the ground before beginning the survey. The pieces of plastic (e.g., old tarps) should approximate the sizes of typical channel units at the site.

3. Calculations

If channel units are measured, average width and depth are calculated according to the following formulas:

$$\text{Average width} = \frac{\text{Width measurements}}{\text{Number of measurements}} \tag{2.2}$$

$$\text{Average depth} = \frac{\text{Depth measurements}}{\text{Number of measurements}} \tag{2.3}$$

Area and volume of each channel unit are calculated as follows. Be sure to use common units.

$$\text{Area} = \text{Length} \times \text{Average width} \tag{2.4}$$

$$\text{Volume} = \text{Length} \times \text{Average width} \times \text{Average depth} \tag{2.5}$$

The percentage of each type of channel unit in the reach, by area or volume, is

$$\% \text{ of Area} = \frac{\text{Area of channel unit type}}{\text{Total area of reach}} \times 100 \tag{2.6}$$

$$\% \text{ of Volume} = \frac{\text{Volume of channel unit type}}{\text{Total volume of reach}} \times 100 \tag{2.7}$$

B. Basic Method 2: Visual Estimation of Channel Units

1. Most channel unit surveys progress in an upstream direction, but this is not essential. It is necessary, however, to be able to recognize channel unit boundaries. These boundaries are often marked by abrupt gradient transitions, which tend to be more easily visible looking upstream than downstream. Begin at a clearly monumented starting point, using GPS if available to establish geospatial coordinates. Starting points are usually located at reach boundaries but may consist of a manmade structure such as a bridge or some other permanent feature of the landscape. If semipermanent markers are used (e.g., a stake or flag tied to a tree), the location of the marker should be precisely referenced.

2. Divide into teams of two or more individuals. Moving along the stream away from the starting point, the team should identify and record each channel unit as it is encountered (Table 2.5). Units located side by side relative to the thalweg (e.g., a pool in the main channel and an adjacent backwater) should be so noted.

3. Record the distance from the starting point of the reach survey to the beginning of each channel unit. This can be accomplished with a measuring tape (or hip chain), rangefinder, or GPS. Unless GPS is used, it will most likely be necessary to measure distances from intermediate reference points along the channel because bends in the channel or riparian vegetation will obscure the view of the starting point. For small streams, it may be helpful to locate intermediate distance reference points at short intervals (e.g., 50 m).

4. For each channel unit, visually estimate the wetted surface area and note it on the data form (Table 2.5). Periodically (e.g., every 10 channel units), use the techniques illustrated in Advanced Method 1 to measure the length and width of a channel unit after its area has been visually estimated. Record these measurements on the data form, as they will be used to determine any systematic bias in the visual area estimates and will make it possible to calculate a correction factor.

C. Advanced Method 1: Detailed Measurements of Channel Units

1. Perform steps 1–3 from Basic Method 2.

2. For each channel unit, measure its greatest length in any direction, and record this length on the data form (Table 2.5). Widths should be measured at right angles to the line defining the greatest length.

3. Measure the wetted width at regular intervals along the length of the channel unit. Although five widths measurements are shown on Table 2.5, the number can vary at the discretion of the investigators. Geomorphically simple units require fewer width measurements than units with complex margins, but in general more is better.

4. If the volume of each channel unit is to be estimated in addition to the area, record the depth of the stream at regular intervals across the channel at each width transect. If the stream is wadeable, depths are usually measured with a telescoping fiberglass surveyor's rod, graduated wading staff, or meter stick (for very small streams). For very large streams, an electronic depthfinder operated from a boat may be appropriate. At a minimum, depth should be determined at one-third and two-thirds the distance from one side of the channel to the other at each width transect, yielding two depth measurements for each width measurement (Table 2.5). Once again, complex channel units require more depth measurements for accurate volume estimates than geomorphically simple units.

IV. QUESTIONS

1. Were preliminary determinations of valley segment and reach types from maps and photographs correct when sites were visited in the field? What types of valley segments and stream reaches would be easy to identify from maps and aerial photographs? What types would be difficult to identify?

2. What would likely happen if each reach type were to experience a very large precipitation event, such as a flood with a 100- to 200-year recurrence interval?

TABLE 2.5 An Example of a Field Data Form for Conducting Channel Unit Surveys. Channel units can be identified by an acronym or alphanumeric designation. Modified from Dolloff *et al.* (1993).

Location_____ Date_____ Surveyors_____

Stream_____ Discharge_____ _____

Quad Map_____ Time_____ _____

Starting Point/GPS_____ Water Temp_____ _____

Ending Point/GPS_____ Reach Type_____ _____

Channel Unit	Distance from Start	GPS Location	Area (estim.)	Greatest Length	Widths					Depths									
					1	2	3	4	5	1	2	3	4	5	6	7	8	9	10

Would the effects be similar to other large disturbances such as inputs of massive volumes of fine sediment?

3. Give a few examples of situations where a stream reach might change from one type to another.

4. How does riparian vegetation influence the characteristics of different reach types? For one or two types, describe how alteration of the riparian plant community could affect channel features.

5. If the channel unit survey compared visual estimates of surface area with estimates derived from actual length and width measurements, was there a tendency for visual estimates to over- or underestimate area? Were errors more apparent for certain types of channel units than for others? Explain why, and suggest a way to correct for systematic bias in the visual estimates.

6. Describe several ways of displaying channel unit frequency data.

7. Describe how the properties of different types of channel units might change with increasing streamflow.

8. Based on your knowledge of the habitat preferences of a certain taxon of aquatic organism (e.g., an aquatic insect or fish species), suggest how that organism would likely be distributed among the channel units within that reach or reaches that were surveyed.

9. How would the frequency of different types of channel units in a reach likely change in response to removal of large wood? To extensive sediment inputs? To destruction of riparian vegetation? To a project involving channelization of the reach?

V. MATERIALS AND SUPPLIES

Field Materials

100 m fiberglass tape or hip chain
Flagging
Global Positioning System (GPS) instrument
Optical or laser rangefinder
Surveyor's rod, graduated wading staff, or meter stick
Waterproof data forms
Camera

Laboratory Materials

Aerial photographs
Geologic, soils, climate, and vegetation maps (as available)
Graph paper
Map wheel (map measure), planimeter, or digitizer
Stereoscope
Topographic maps

VI. REFERENCES

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Discharge Measurements and Streamflow Analysis

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I. INTRODUCTION

One of the most important of all the geologic processes is the force applied to land forms by running water. In the same manner, running water can have a significant effect upon the distribution of the flora and fauna in lotic ecosystems (see reviews by Statzner *et al.* 1988, Gordon *et al.* 1992, Allan 1995, and Gore 1996). The most fundamental of hydrologic measurements that characterize all river and stream ecosystems is that of *discharge*, the volume of water flowing through a cross section of a stream channel per unit time. The amount of water flowing through a reach of channel, combined with the slope of the stream channel, yields an indication of *stream power* or the ability of the river to do work. The potential energy lost in the reach is dissipated as frictional heat loss on the streambed and when the stream picks up and moves material. The work performed by the stream is important to lotic ecologists because it influences the distribution of suspended sediment, bed material, particulate organic matter, and other nutrients. The distribution of these materials has substantial influence on the distribution of riverine biota by altering physical habitat conditions (redistribution of substrate) and the availability of energy (as measured by primary production or the state of particulate organic matter) (Vannote *et al.* 1980, Vannote and Minshall 1984, Statzner *et al.* 1988). In addition, discharge and stream power combine with other basin conditions to influence meander pattern and floodplain dynamics (Leopold *et al.* 1964, Hornberger *et al.* 1998, Dorava *et al.* 2001). Civil engineers and water managers use these same relationships

to create catchment water budgets, to determine the feasibility of electricity production through hydropower generation, and to estimate of the ability of a river system to absorb or process chemical contaminants (Lee and Lin 2000).

Traditionally, discharge has been measured in the United States in terms of cubic feet per second (cfs), but with greater emphasis on employing SI units, most ecologists prefer to express discharge as cubic meters per second (m^3/s or cms) (sometimes called *cumecs*). On the other hand, water resource managers often express discharge in consumptive terms as millions of gallons per day (mgd). Because so much of the hydrological information in the United States is maintained by the United States Geological Survey (USGS), stream ecologists and hydrologists must be familiar with translating gaging records. This conversion can be expressed as $1\,cfs = 0.028\,m^3/s$ (cms) and $1\,m^3/s$ (cms) $= 35.315\,cfs$. Similarly, one cubic foot is approximately 7.48 gallons. Thus, $1\,cfs = 7.48$ gallons US/s (0.646 mgd).

Discharge in streams and rivers can vary annually from a few cubic feet per second in headwater streams to seasonal variations from 1.5 million to over 12 million cfs in the Mississippi River, near New Orleans. More infrequently, dramatic variations (in the form of rare flood events) may occur. A greater than fourfold increase in peak discharge (some 200,000 cfs [5664 cms]) occurred on the Colorado River, through the Grand Canyon, in 1921. The highest river discharge ever recorded was 52.5 million cfs [∼1.5 million cms] on the Amazon River in Brazil (Cech 2003).

At most gaging stations, flow is measured by recording the *stage*, or height, of the surface of the water above an arbitrary datum (or benchmark). Using discharges calculated for a nearby cross-section at different velocities and water surface elevations (or stages), a graphical relationship (Figure 3.1) between stage and discharge produces a *rating curve* so that discharges can be predicted at stages other than those measured. Although many measured points are more accurate, it is not uncommon for rating curves to be based on three-point regressions: a low flow, a median flow (near base flow), and a high flow. Although the USGS most commonly uses a *stilling well* and microwave-relayed information to provide instantaneous gaging data, a simple *staff gage*, a piece of metal rod with measured increments representing measures of stage height, is often used at each sampling site so that stream ecologists can quickly note discharge at any observation time. Note that hydrologists often refer to "the stage at zero flow." This is not the period when the channel is dry, but rather it is the stage or water surface elevation at which the effective discharge measured across the given transect is 0 cfs or cms. An easy way to approximate this stage at zero flow is to measure the elevation of the lowest point at the controlling cross-section.

An analysis of the manner in which discharge varies over time, or the *hydrograph*, allows a lotic scientist so examine the characteristics of the watershed that influence such conditions as runoff and storage. A hydrograph (Figure 3.2) can be plotted from gaging records to display yearly, monthly, daily, or instantaneous discharges. Ecologists, hydrologists, and water resource managers usually obtain gaging records from *Water Supply Papers*, published annually by the Water Resources Division of the USGS. By standard convention, and based on the average time that flows are lowest in the United States, *Water Supply Papers* are published by *water year* (e.g., Water Year 2006 runs between October 1, 2005 and September 30, 2006). These data can provide information on total flow (monthly and daily), mean monthly discharge, base (often groundwater maintained) flow, stage height, and periods of high and low flow. *Water Supply Papers*, as well as instantaneous gage readings, can also be obtained, via Internet access, at http://waterdata.usgs.gov/nwis/sw. The website contains many options for data format and even contains a tutorial on how to retrieve data and interpret the results.
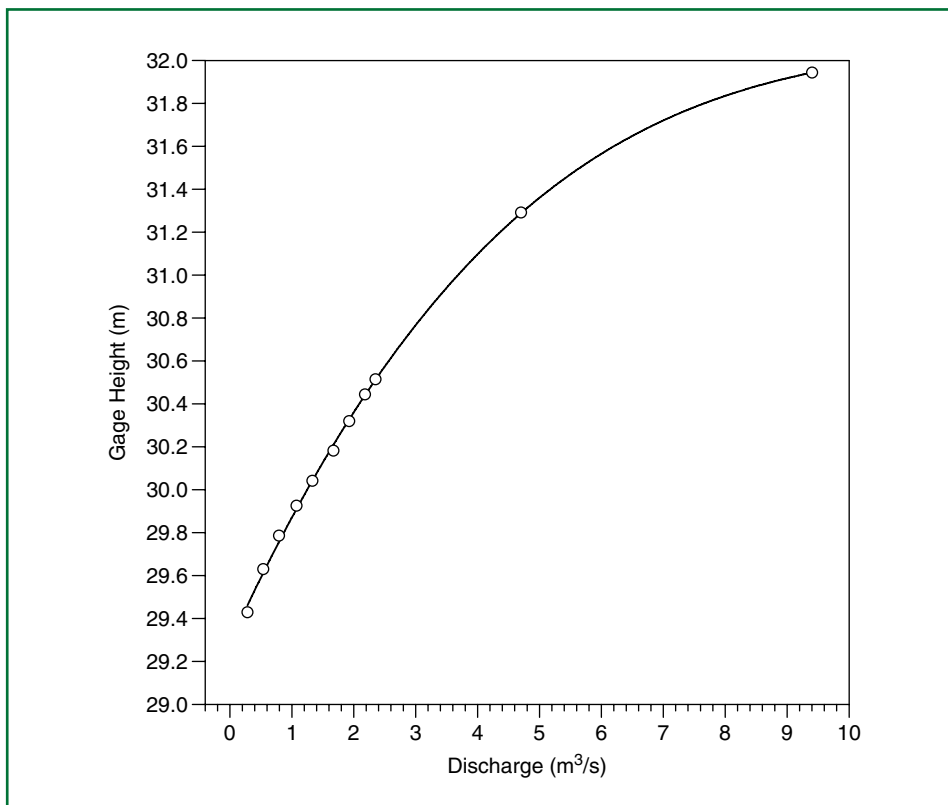
FIGURE 3.1 Stage-discharge relationships for the Olifants River (near Hexrivier Farm, Eastern Cape Province, Republic of South Africa). Based on surveyed water surface elevations (gage height) and discharge calculated using a current meter.

In general, hydrographs of rivers in temperate mountainous areas, or regions with major contributions from mountain catchments, will be dominated by spring snowmelt and will show a major peak during the spring. On the other hand, those rivers in lowland temperate and subtropical systems undergo multiple flood events of varying intensity and duration over the course of a water year. Figure 3.2 depicts some of these differences. Any peak in a hydrograph is termed a *flood or freshet*, regardless of whether it overtops the banks of the channel. When there are no flooding events, baseflow is provided by inflow from groundwater and storage within the catchment. The baseflow level will fluctuate as recharge from precipitation occurs. Examination of the shape of a daily hydrograph during a storm event can indicate the condition of the stream and its basin (Figure 3.3). The rising limb of the curve is usually concave and is an index of infiltration capacity of the catchment. In a small basin, the time from the onset of precipitation to the rise in the ascending limb of the hydrograph represents the time to reach soil saturation and for runoff to collect at the point of measurement. A catchment with a large storage capacity, absorptive surface, or large channel will have a lower stage-height peak than a similar-sized basin with little storage (e.g., small channel, lower vegetation density, more clay in soils, greater human development [more impermeable surfaces], etc.). Agricultural
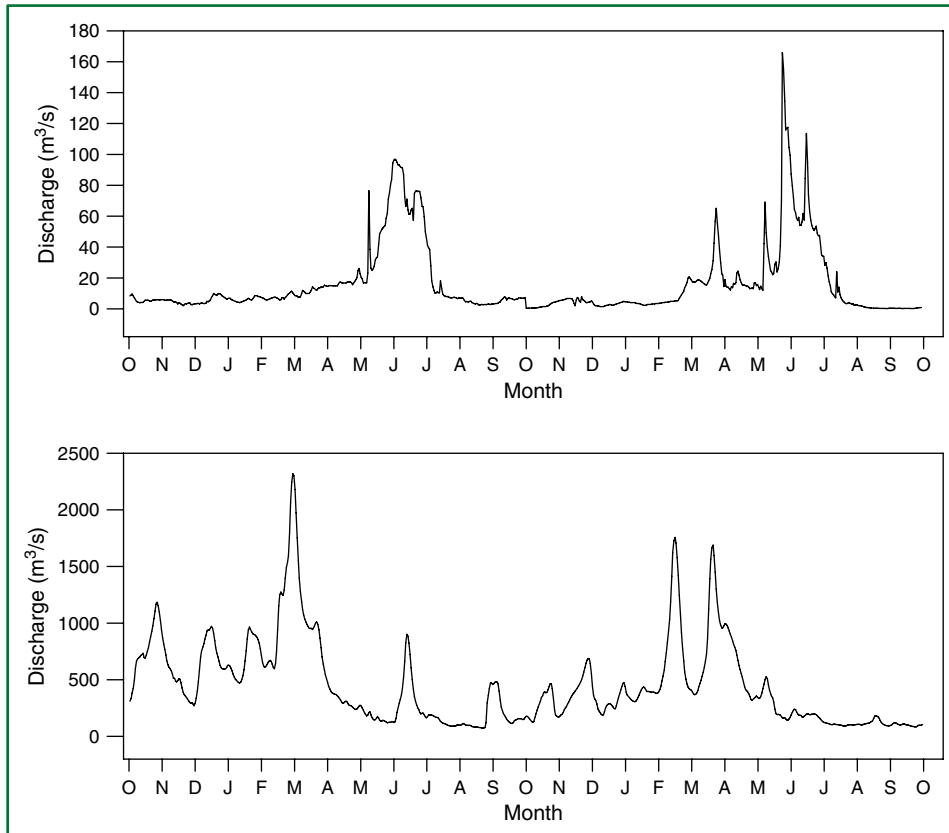
FIGURE 3.2 Hydrographs (based upon daily discharge readings) of the Tongue River (near Miles City, Montana) for water years 1965 and 1966 and the Altamaha River (at Doctortown, Georgia) for water years 1996 and 1996. The Tongue River is a snowmelt-dominated hydrograph while the Altamaha River is flashy, being dominated by rainfall events.

land, for example, produces a more rapid response in the hydrograph than woodlands because densely wooded areas restrict surface flow and enhance infiltration (Gregory and Walling 1973). The shape of the hydrograph also reflects the longitudinal profile and basin shape. A steep basin gradient is reflected in a rapid response curve, whereas a low-basin gradient will produce a hydrograph with a slow and prolonged response curve. A catchment with many headwater streams but few tributaries in lower reaches will produce a hydrograph with a sharp peak flood. However, the peak is delayed from the onset of the precipitation event. An elongated channel with many tributaries has a hydrograph that rises rapidly and falls over a long period of time. A catchment with many subbasins often produces a hydrograph with several flood peaks, depending on distribution of rainfall in the area.

Analysis of the flood hydrograph can reveal some qualities of the catchment basin itself. For example, there exists a strong correlation between the number of days between flood peak and the end of runoff (return to baseflow) (D) *and* the area of the catchment
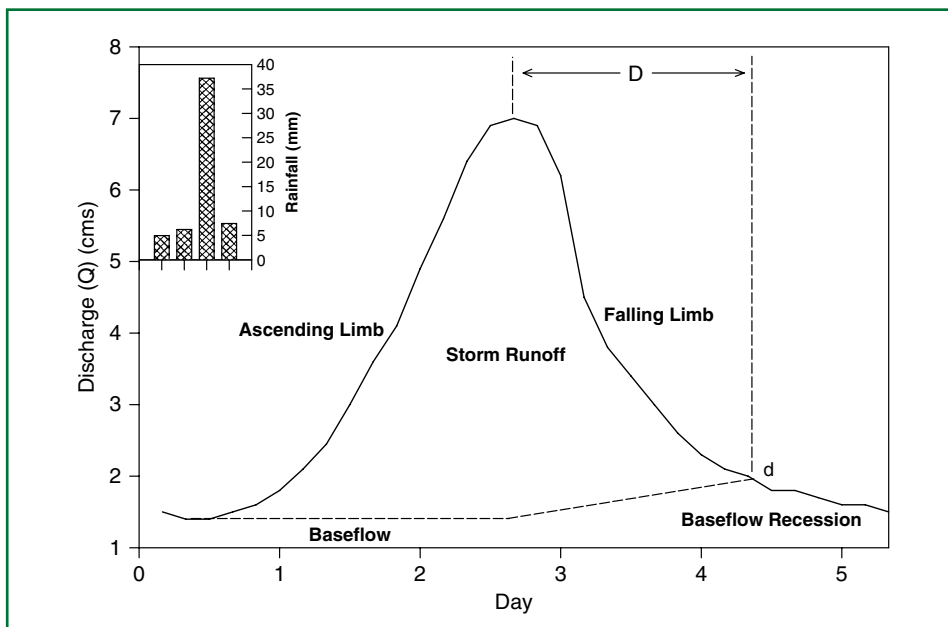
FIGURE 3.3 A hypothetical flood hydrograph illustrating the delay between the precipitation event and the actual flood event. Point "d" is the point at which surface runoff from the flood has ended and discharge (baseflow) is maintained by groundwater inflow. "D" is the time interval between the peak of the flood and the return to baseflow.

basin. Chow (1964) reported a standard method for estimating the end of the runoff period as a function of the catchment area (A) (in km^2):

$$D = 0.827 A^{0.2} \tag{3.1}$$

The decline in groundwater input to a stream is known as *groundwater recession* and reflects the drought condition of the catchment.

The discharge of a stream or river is also affected by conditions within the channel and the channel geometry. The location of that portion of the channel that carries the greatest portion of flow (usually the deepest part of the channel), the *thalweg*, is influenced by the shape of the banks, the width of stream, the bed material, and the rate of deposition of sediment. In general, the highest stream velocities occur at or near the *thalweg* (see Chapter 4) and are a function of resistance to flow, usually as a result of streambed material (i.e., bed roughness). The *wetted perimeter* is the cross-sectional distance along the streambed and banks where they contact water. Wetted perimeter can be the same for a deep, high-banked, narrow mountain stream and a broad, shallow lowland river, yet the same discharge through those channels will yield very different flow conditions (Lane 1937, Chow 1959). The *hydraulic radius* of the stream is the ratio of cross-sectional area to the wetted perimeter of the flow at the surface. In streams that are wide in relation to their depth (e.g., greater than 20:1, width: depth), hydraulic radius and hydraulic depth

are nearly equal and are approximated by the average depth of the stream because the wetted segment on the banks is small compared to the wetted length of the bed. Most hydrologists and stream ecologists, thus, use the terms "mean depth," "hydraulic depth," and, sometimes, "hydraulic radius" interchangeably.

In addition to information about water supply to water resource managers, discharge data are most often used to make predictions about the duration, intensity, and probability of flood events. These predictions are accomplished by the production of flow-duration curves and flood-frequency predictions. A *flow-duration curve* is a semilogarithmic plot of discharge versus the percentage of time that a given discharge is equaled or exceeded (Figure 3.4). If the curve has an overall steep slope, the catchment rapidly captures a large amount of direct runoff. If the curve is relatively flat, there is substantial storage within the catchment, as surface or groundwater and runoff collects more slowly (Morisawa 1968). A frequent application of discharge records is to predict the magnitude and frequency of flood events. The *flood-frequency curve* allows hydrologists to assess the probability of a certain sized flood or greater occurring in any given year. By convention, maximum discharges for each year of gaging record are ranked and plotted as a cumulative frequency curve. A recurrence interval (the number of years within which a flood of a
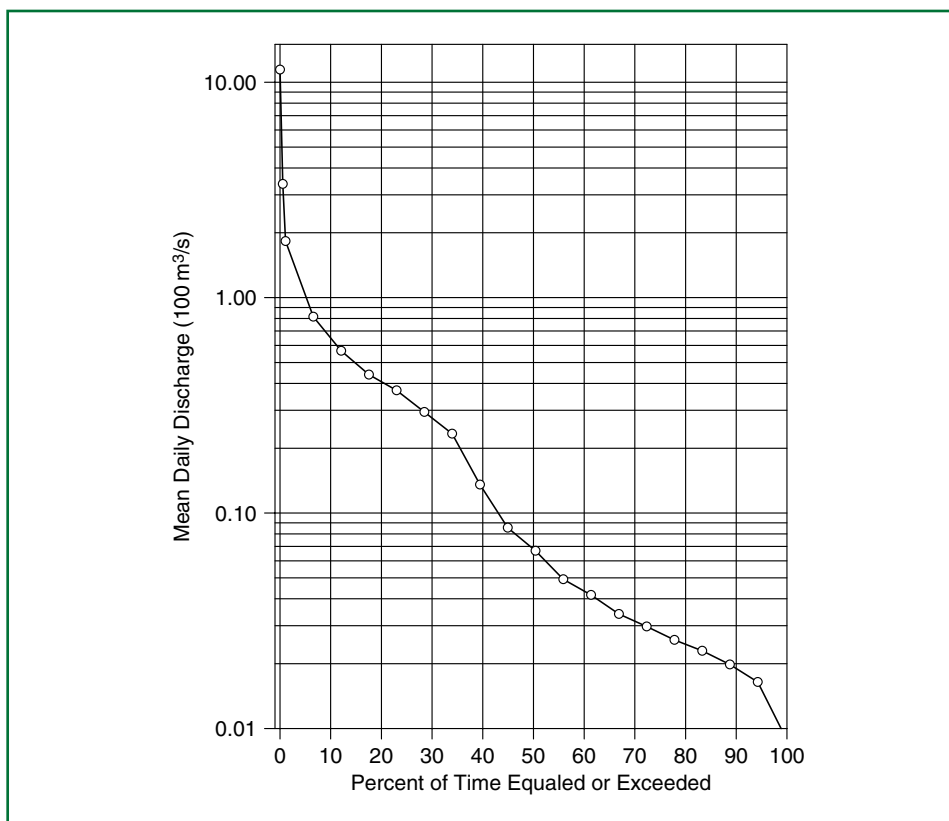
FIGURE 3.4 Flow duration curve for the Locust Fork River, at the USGS gaging station near Trafford, Alabama, for water year 1951.
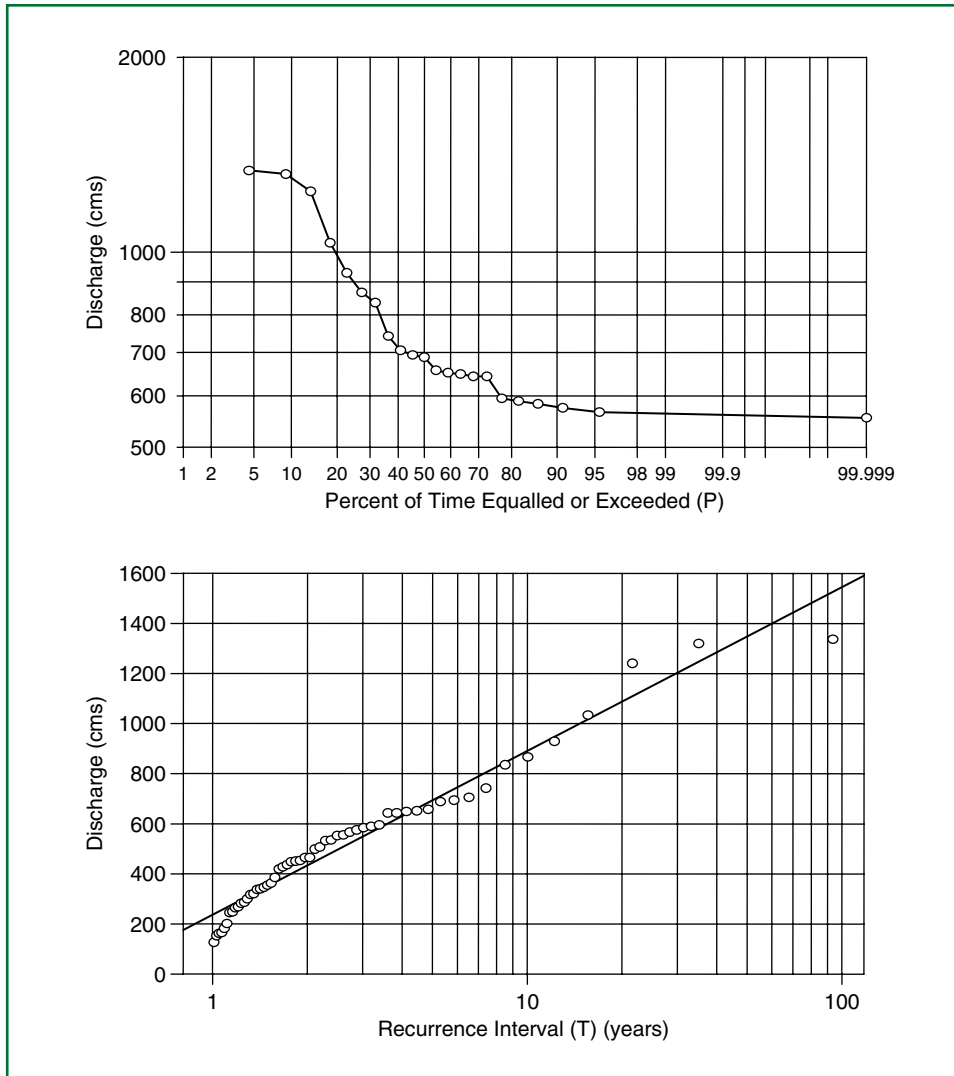
FIGURE 3.5 Flow duration curve and recurrence interval for the Saco River, at the USGS gaging station near Conway, New Hampshire, between water years 1930 and 2002. Note that the predicted 100-year flood is near 1600 cms.

given magnitude or greater is likely to occur) may be calculated as an alternative way of expressing flood frequency (Figure 3.5).

Another useful representation of flows may be obtained by plotting the cumulative discharge versus time. This allows the actual sequence and persistence of flows from month to month or year to year to be assessed. The slope of the flow line in this form of plot (a mass curve) is equal to the rate of flow (Figure 3.6). When plotted over a period of many years, for example, one can tell the magnitude and frequency of a certain discharge in successive years. When fish population data are available, for example, it is possible to correlate year-class success with the magnitude and duration of flows during spawning and incubation to get an estimate of a minimum flow required to maintain
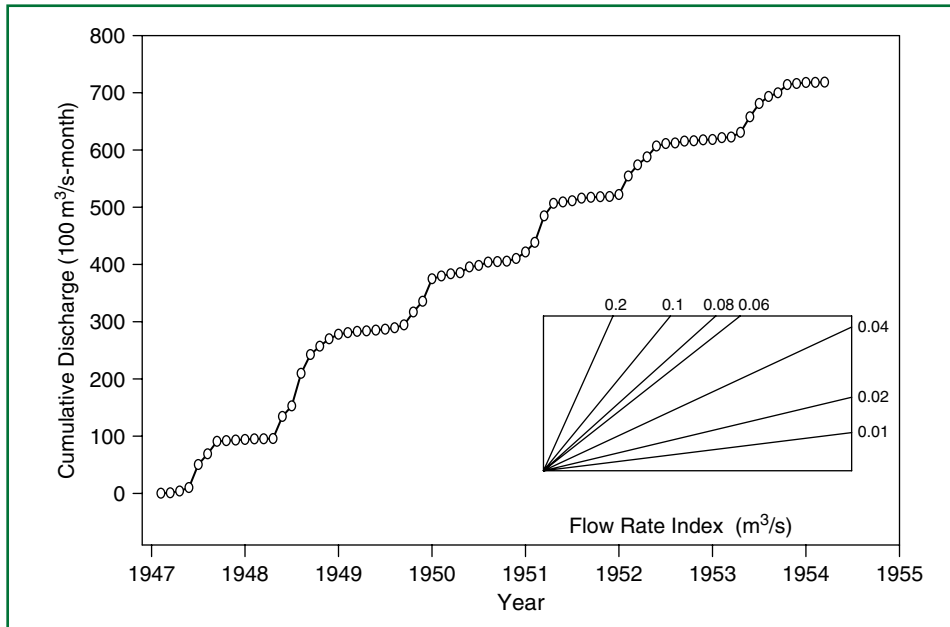
FIGURE 3.6 Discharge mass curve for the Locust Fork River, at the USGS gaging station near Trafford, Alabama, for water years 1947 through 1954.

population stability (Newbury and Gaboury 1993). When the discharge calibration curve is translated into storage capacity (say, acre-feet per year), tangents drawn at the high points on the mass curve can yield information about optimal reservoir size and/or the yield of a given catchment (Linsley *et al.* 1992).

In this chapter, several field methods to measure discharge are presented along with analytical techniques to produce and examine hydrographs. Some methods are appropriate only for low-order streams, but most can be adapted to larger order systems. The specific objectives are to: (1) understand methods used to pick a specific site for discharge measurement; (2) familiarize lotic researchers with the proper techniques for using current meters, calculating velocities and discharges, and producing and analyzing hydrographs; and (3) provide a better understanding of the use of discharge analysis to interpret channel form, basin shape, land-use patterns, flood conditions, and the distribution of biota in the river system.

II. GENERAL DESIGN

Discharge is usually determined by multiplying the mean velocity by the cross-sectional area of the flow. The cross-sectional area can be measured directly by stretching a measuring tape across the stream or river (this is modified in large rivers by using premeasured cables or surveying techniques) and taking several measurements of depth with a meter stick or surveyor's leveling rod or staff. Several measurements of mean velocity must be taken across the stream, because flow is unevenly distributed across the stream channel. However, if the flow is very irregular, say on a meander bend or where undercut banks and boulders obstruct or alter flow, the entire velocity distribution

must be measured and plotted to determine a mean. In general, stream ecologists and hydrologists try to avoid these situations because of the relative difficulty in obtaining accurate measures at these sites. Between entering tributaries, discharge should be fairly constant but may vary with gains or losses to the stream channel. In alluvial, gravel-bed streams, a significant amount of water may be lost to or gained from the hyporheic zone along an unconfined stream reach. Likewise, measures of true discharge may vary according to the sensitivities of the equipment and abilities of the researchers. A useful tactic is to measure the discharge across several transects in a stream reach and, then, to compare the calculated flows.

A. Site Selection

The selection of the site for measurement of discharge is a critical consideration. In general, the best sites are those in which the flow appears to be relatively uniform across the width of the channel and the surface is not broken by protruding objects, which tend to alter local velocity and depth measurements. The selected section should then have uniform flow that is parallel to the banks. With these stipulations in mind, it should not be surprising that the USGS most often calibrates their gaging stations by measurements at or near bridges built across a river. Bridge engineering requires that the channel be modified to a have a relatively uniform depth in order to accommodate the bridge; thus, providing the criteria for discharge measurement. In low-order streams or those streams with very low discharge (usually with nonparallel or sinuous flow patterns), a small straight section of channel (essentially, a small weir) can be built up using large stones between which the majority of flow passes. This system can then be used to measure discharge; however, in either case of a constructed weir or bridge readings, measurements cannot be used to describe the pattern of flow. That is, measurements from an artificial section should not be reported as typical mean depths and velocities.

Generally, volumetric analysis (Basic Method 1) is most appropriate for small streams (first- and second-order). The velocity-area method (Basic Method 2) is appropriate for any stream order, but it works best on third-order and higher systems. Under unusual flow conditions (extremely shallow, low flows or bankfull or overbank floods), the slope-area method (Advanced Method 1) can be most useful in estimating discharge. Analysis of discharge patterns over a long period of time is best accomplished in the field by establishing a stage-discharge relationship (Advanced Method 2) and through graphical and mathematical analysis of published gaging records (Advanced Method 3).

B. Discharge, Cross-Sectional Area, and Velocity

The simplest form of discharge measurement is:

$$Q = Av \tag{3.2}$$

where Q represents discharge (in cfs or cms); A, the cross-sectional area of the channel at a certain transect; and v, the mean water-column velocity at a designated transect. (Note: Many European hydrologists use the term U rather than v to denote mean water column

velocity.) Cross-sectional area and mean velocity can be obtained by using a control structure (such as weir) or incremental measurements across a transect of known width.

Weirs

The use of a weir to measure discharge is based upon analysis of flow conditions as it passes over the crest of the weir. The simplest configuration occurs when a sharp-crested weir impounds a pool of still water. As the flow passes over the crest, it achieves critical velocity (Fr=1, see Chapter 4) where the critical depth of flow is two-thirds of the height of the pool above the weir crest. The critical velocity is directly related to the critical depth, so measurement of the height of water in the pool behind the weir can be used to determine the discharge in the channel (Figure 3.7) The derivation of these relationships from the Bernoulli equation is fairly simple and fully explained by Hornberger *et al.* (1998).

The depth of the water pooled behind the weir (h_{weir} m) is thus related to the depth of the water at critical flow over the weir (h_0 m), as:

$$h_{weir} = \frac{3}{2} h_0 \tag{3.3}$$

At the point on the crest where flow is critical (Froude Number = 1), velocity, v, is equal to the square root of the product of gravity, g (9.8 m/s^2) and h_0. Thus, the velocity over the crest of the weir (v_0 m/s) is:

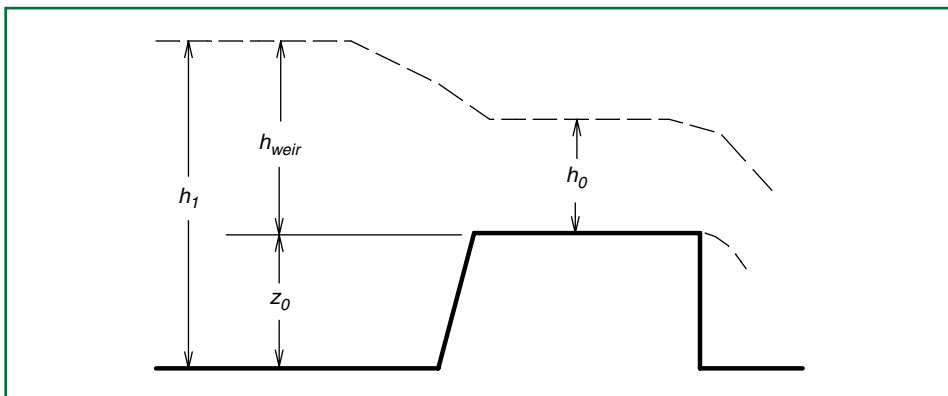$$v_o = \left(\frac{2}{3} g h_{weir} \right)^{1/2} \tag{3.4}$$

FIGURE 3.7 Cross-section schematic of a broad-crested weir. As described in the text, discharge measurement is dependent upon measurements of h_{weir} ($h_1 - z_0$). Note: The depth of flow is adjusted so that h_0 is 2/3 of h_{weir}.

For a wide channel where little contraction of the flow occurs, if the "across-channel width" of the weir crest is (w_c), the discharge is:

$$Q = v_0 w_c h_0 \tag{3.5}$$

Thus, it is possible to measure something relatively simple (h_{weir}) to obtain an estimate of discharge (Q), which is a reasonably difficult task using other methods. The equation presented for the sharp crested or broad-crested weir can not be used for other types of weirs as the terms inside of the brackets will change. Equations for discharge measured through V-notch weirs, rectangular-notch weirs, and other control structures are commonly found in any text on hydrology or environmental engineering (Lee and Lin 2000).

Midsection Method

The midsection method is a standard technique used by most hydrologists (and recommended by the USGS) for calculating the discharge of most streams and rivers (Figure 3.8). To measure discharge (Q), stretch a measuring tape (or a tag-line or a nylon or braided cable with permanently marked intervals) across the stream and then divide the transect into n convenient increments, or *cells*. In fact, the observation point locates the center of the cell to be examined with cell boundaries halfway to the next observation point. If the flow is relatively uniform, the transect should be divided up into at least five cells of equal width. As a general rule, however, cell widths should not exceed 3 m. A stream of 30 m width, then, should have at least ten observation points (or verticals). It is not necessary to make uniform width cells. If there are any hydraulic irregularities (a protruding boulder, a cascade, a pool, etc.) across the transect, a new cell or observation point should be designated where more uniform conditions resume. If the flow is uniform, the mean velocity is measured at the observation point at a height above the stream bed equal to 0.4 times the depth at that location (see Chapter 4 for the reasoning behind this choice). At an observation point where the depth exceeds 60 cm, the mean velocity should be calculated as an average between velocities measured at 0.2 and 0.8 times the depth at the observation point.

The partial discharge for cell x is computed as:

$$q_x = v_x \left[\frac{\left(b_{(x+1)} - b_{(x-1)} \right)}{2} \right] d_x \tag{3.6}$$

where
   q_x = discharge through partial section x
   v_x = mean velocity at observation point x
   $b_{(x-1)}$ = distance from the datum to the preceding observation point ($x-1$)
   $b_{(x+1)}$ = distance from the datum to the next observation point ($x+1$)
   d_x = depth the water at observation point x.
   The total discharge (Q) is the sum of the partial section discharges (q_n).
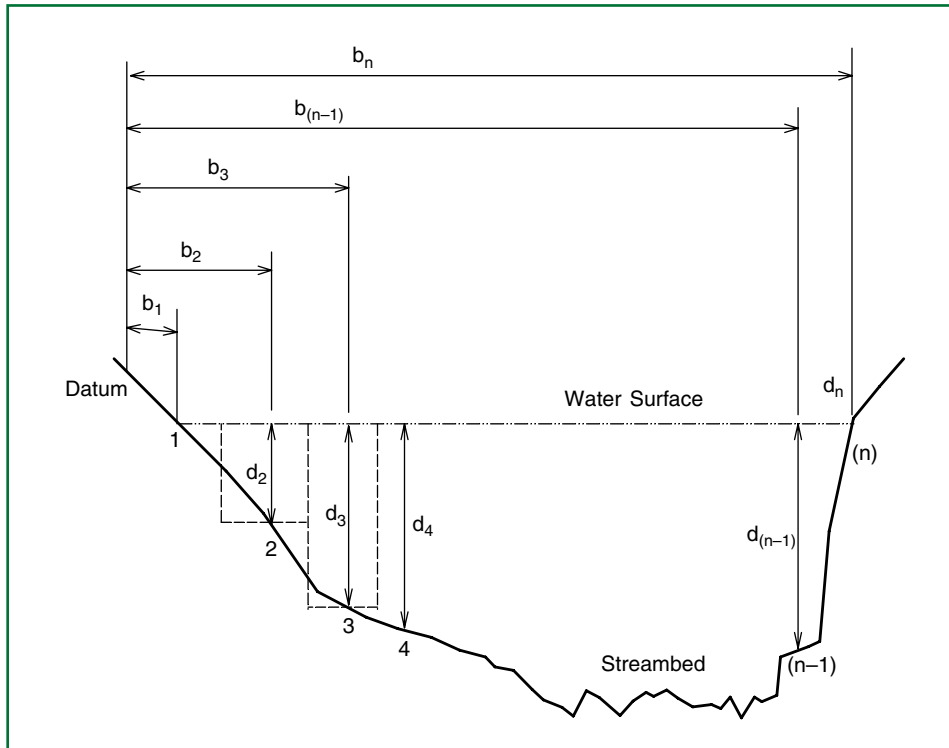
FIGURE 3.8 Schematic of the midsection method for computing cross-sectional area for discharge computations. 1, 2, 3, ... n are observation points. $b_1, b_2, b_3, \ldots b_n$ are distances (transect intervals) from the datum to the observation points. $d_1, d_2, d_3, \ldots d_n$ are depths of water (surveyed verticals). The dashed lines outline the section (cell) being measured.

Current Meters

There are a variety of velocity meters available and all are acceptable for most measurements using the midsection method. Each requires its own special technique for use. In general, most hydrologists prefer either mechanical (pygmy, Price AA, or Ott) or electromagnetic (Marsh-McBirney) meters. In large or great rivers, where widths may be over 100 meters or depths greater than 30 meters, a laser-profiler may be the only way to effectively measure current velocities, since stringing tag-lines and suspending current meters from extremely long and heavy cables is impractical.

Mechanical current meters are the most widely used by practicing hydrologists and ecologists. Mechanical meters are of two main types; those having vertical-axis rotors (bucket-impeller) (Price type AA or pygmy type current meters) or horizontal-axis rotors (with vanes) (Ott-type). Each has unique advantages as described below.

Vertical-axis (type AA or pygmy):

1. Operates in lower velocities than horizontal-axis meters.
2. Bearings are well protected from silt-laden water.
3. Relatively easy to repair in the field.
4. Single rotor can serve over the entire range of velocities.

Horizontal-axis (Ott):

1. Rotor disturbs less flow than do vertical-axis types because of the axial symmetry with the direction of flow.
2. Rotor is less likely to be entangled by debris, periphyton, etc.
3. Bearing friction is less since no horizontal pressure is applied to the axis of rotation.

As with electromagnetic current meters, the horizontal axis current meters require more frequent calibration than the vertical rotor meters. However, the distinct advantage of the electro-magnetic current meters is that they are capable of measuring negative velocities (that is, eddy or lateral flows).

Depth Measurement (Sounding)

The current meter is attached to a stainless steel wading rod is marked in increments of 0.1 m or 0.1 ft. There two commonly used types of wading rods; the top-setting rod and the round rod. The top-setting rod has preferred because of the convenience of setting the current meter at the correct depth, using a Vernier scale on the handle (or the *setting rod*), and keeping the hands dry. When the setting rod is adjusted to the depth of the water, measured on the graduated main rod, the current meter is automatically set at the correct depth. Using the setting rod to place the current meter at twice the depth and half the depth will give readings of $0.2 \times$ the depth and $0.8 \times$ the depth, respectively. In large rivers, a winch and cable in conjunction with a sonde replaces the wading rod. The winch is attached to a "bridgeboard" that is suspended on a bridge over the river (transect intervals being temporarily or permanently marked on the bridge railing). If there is not a convenient bridge across the large river, the winch can be attached to a bridgeboard mounted to the front of a stable boat, which is tethered to a cable stretched across the river. This cable stabilizes lateral movement across the transect and can also serve as the tag-line. On large rivers, this can become a danger to boat traffic and all care must be taken to not only flag the line with bright colored markers but to place personnel upstream and downstream along the river to warn approaching boat traffic. In either case, the current meter is attached to the end of the cable. The winch, which usually has a depth meter attached, is calibrated to a zero point when the current meter is at the surface of the water. When using the cable, a finned weight (up to 50 kg), sometimes called a *sonde*, is attached below the current meter to maintain both vertical position on the transect and orientation into the current.

When using a mechanical current meter, a set of earphones is employed to "count clicks" (in the earphone) produced by each revolution of the current meter. The count should last for a minimum of 30 seconds and the exact number of revolutions and the time counted should be recorded for each cell. Note that the number of revolutions should be recorded as one less than the number of "clicks" counted, since the first click does not signify a complete revolution of the rotor of the current meter. Many newer instruments have automatic timing devices and internal calculators that will provide a direct digital readout of the velocity. If no such electronic readout is available, an instrument rating-curve, provided by the manufacturer, should be consulted to yield a velocity measured by each revolution.

As previously noted, the product of the width of the cell, the depth at the midpoint of the cell, and the mean velocity is calculated as the cell discharge. The sum of the cell

discharges, then, is the discharge for the stream on the date at that stage height (or water surface elevation).

Acoustic Doppler Current Profiler (ADCP)

As mentioned earlier, the logistics of transect measurements using a tag-line or cable, not to mention boating and personnel safety concerns, makes discharge measurements on large or great rivers nearly impossible with standard techniques. This is particularly true when discharge measurements must be made in rivers with unsteady flows (say, from variable hydropower releases) or tidally influenced streams, where discharge can vary over 100% in a 10-minute period. This difficulty has been overcome in recent years with the development of a sonar-related device, the acoustic Doppler current profiler (ADCP) (Morlock 1996, Simpson 2001). ADCP can be used without the necessity of moving at intervals along a transect defined by a cable or other straight-line device across the channel. The ADCP is suspended from a boat as it moves across the river and software combines geographical position system (GPS) data with boat movements and velocities and the sonar signal to acquire velocity readings in the water column.

An ADCP applies the Doppler principle by bouncing ultrasonic sound pulses off small particles (sediment and other organic material) (called *backscatter*) suspended in the water column. Even in optically clear waters, there is still enough suspended material to allow ADCP to be utilized. It is rare that backscatter density is so low that ADCP cannot be used. The software accompanying the ADCP measures the Doppler shifts as the boat moves across the river and, relative to boat speed, direction relative to current flow and concurrent backscatter movement calculates and displays velocities in the water column. Velocity measurements at intervals as small as 0.2 mm, from surface to substrate, have been obtained. As one might imagine, this is not a simple or inexpensive methodology. The boatman must train to move laterally across the channel yet keep the boat pointed upstream as much as possible in order to obtain the full component of Doppler shift. Currently, this is not an "off-the-shelf" technique and is continually being modified and tested. To be most effective, the operator must have a rudimentary knowledge of acoustic physics, knowledge of the workings of the specific ADCP system, the software system, as well as proper boating techniques. Simpson (2001) describes the latest USGS recommended practices for ADCP operation and data interpretation.

The ADCP system usually utilizes a downward-looking profiler that broadcasts a forward, aft, and right- and left-lateral acoustic signals, each angled approximately 30° from the vertical transducer (called a *Janus* configuration). The velocities measured at each depth are the center-weighted mean of velocities measured throughout the entire acoustic sampling window. To measure absolute water velocities, the ADCP must be able to sense and measure the velocity of the equipment relative to the substrate (*bottom tracking*). So, simultaneously, the ADCP is tracking the depth of the bottom, and boat speed and direction relative to the substrate. In order to calculate discharge, the cross-product velocities are integrated over the water depth and, then, integrated, by time, over the width of the cross-section. The general equation for determining discharge through a surface, *s*, is modified as:

$$Q_t = \int\limits^{T} \int\limits^{d} \left(\left(V_f \times V_b \right) k \right) dz \cdot dt \qquad (3.7)$$

where

Q_t = total river discharge (cms)

V_f = mean water-velocity vector, in meters/second

V_b = mean vessel-velocity vector, in meters/second

k = a unit vector in the vertical direction

d_z = vertical differential depth (meters)

d_t = differential time (seconds)

(Simpson and Oltmann 1993)

Although the ADCP is most commonly mounted on an arm (swiveling or permanently affixed) attached to a boat, remote-controlled robot boats and sleds suspended from bridges have also been used in certain applications.

Incorporating Channel Resistance and Slope

For a variety of purposes, such as more sophisticated hydrological and ecological modeling, it may be necessary to incorporate changes in streambed roughness or changes in gradient as they affect discharge over the length of the stream. These conditions, especially roughness, can alter velocity significantly (see Chapter 4). As a result, equations that incorporate resistance to flow have been developed to estimate the average velocity of the flow. Roughness is evaluated in a number of different ways, as described below. Energy slope (S) is calculated as the change in elevation and kinetic energy over a given distance. In a stream with uniform flow, this can be measured as the change in elevation of the water surface. If the reach can be located on a topographical map (or GIS system), valley slope can also be estimated. A far better, but more time-consuming method is to take water surface elevations at a point three channel-widths upstream and three channel-widths downstream of the transect using a leveling rod and a surveyor's level to estimate change in elevation over that short distance. Six channel-widths usually encompass the slope changes in pools, riffles, and runs in alluvial rivers to derive an average value.

Chezy's Equation was developed in the 1700s and incorporates channel roughness (C) to estimate stream discharge. The equation is:

$$V = C\,(RS)^{1/2} \tag{3.8}$$

where R is the hydraulic radius (in meters) and S is the energy slope (Henderson 1966). Chezy's C varies from approximately 30 for small, cobble-bottomed streams up to 90 for large, smooth sand-bottomed rivers (White 1986). Discharge is calculated as the product of the cross-sectional area (A) and the calculated velocity (V) value (see Eq. (3.1)). Chezy's equation is used primarily in Europe. The details for calculating C are described by Chow (1959) and Chanson (1999).

Manning's Equation is more commonly used for calculations of discharge where bed roughness is of great concern. It is expressed as:

$$V = \frac{1}{n}\left(R^{2/3}S^{1/2}\right) \tag{3.9}$$

or

$$Q = \frac{1}{n} \left(AR^{2/3} S^{1/2} \right) \tag{3.10}$$

where n is an index of roughness known as "*Manning's n.*" The standard technique for approximating Manning's n for high and moderate flows is presented in Table 3.1. It should be noted that calculating discharge according to Manning's equation does not require direct measurement of average velocities but instead depends on reliable and

TABLE 3.1 Calculation of *Manning's "n"* from Field Observation [Adapted from Cowan (1956)].

$$n = (n_0 + n_1 + n_2 + n_3 + n_4)\ m$$

Additive Factors	
Material Involved	n_0
Earth	0.020
Rock Cut	0.025
Fine Gravel	0.024
Coarse Gravel	0.0028
Cobble	0.030–0.050
Boulder	0.040–0.070
Degree of Irregularity	n_1
Smooth	0.000
Minor (slight scour)	0.005
Moderate (slumping)	0.010
Severe (eroded banks)	0.020
Variation in Channel Cross Section (location of *thalweg*)	n_2
Gradual	0.000
Alternating Occasionally	0.005
Alternating Frequently	0.010–0.015
Effect of Obstructions	n_3
Negligible	0.000
Minor (15% of area is turbulent)	0.010–0.015
Appreciable (up to 50% is turbulent)	0.020–0.030
Severe (>50% is turbulent)	0.040–0.060
Riparian Vegetation	n_4
None	0.000
Low (grass/weeds)	0.005–0.010
Medium (brush, none in streambed)	0.010–0.025
High (young trees)	0.025–0.050
Very high (brush in streams, mature trees)	0.050–0.100
Multiplicative Factors	
Degree of Meandering	*m*
Minor	1.000
Appreciable	1.150
Severe	1.300

consistent evaluations of the channel condition and an accurate measurement of the cross-sectional area, hydraulic radius, and slope. All of the channel resistance equations must be applied with caution at very low flows. Illustrated guides to roughness values and discharge are often useful (see Hicks and Mason 1991).

Flow-Duration Analysis

Flow-duration analysis curves can be prepared only if gaging records for single locations on a stream are available for a substantial period of time, usually several years. In the United States, gaging records can be obtained through local offices of the USGS. Otherwise, a gaging station or a staff gage that has been calibrated and read at regular intervals must be installed to generate the flow data.

To prepare the flow duration curve, all flows during the given period (i.e., daily, monthly, or yearly, depending upon the analysis needed) are listed according to their magnitude. The percentage of time that each discharge was equaled or exceeded is then calculated and plotted on a semilogarithmic plot (percentages on an arithmetic scale on the x-axis and the log of the discharge on the y-axis). Analysis of the shape of the curve provides an idea of basin or catchment characteristics. Searcy (1959) provided a manual of duration curve interpretation. Several duration indices have been used to compare various stream systems. For these purposes, the same period of record must be used for production of all flow duration curves. The discharge at which flows are exceeded 50% of the time is the median flow, or Q_{50}. The Q_{90} is often used as a low-flow (or minimum flow) index. The ration Q_{90}/Q_{50} is often used as an index of baseflow contribution (Gordon *et al.* 1992), whereas Q_{10}/Q_{50} may be used as an index of flood peaks. At the very upper range of discharges, the values between Q_{30} and Q_{10} have been used to analyze the value and importance of the floodplain by the amount of time it is under water.

Flood-Frequency Analysis

The Weibull plotting method is the most commonly applied technique for analyzing flood conditions (Dalrymple 1967). To construct a recurrence curve, the average daily flows are most often examined. When producing a flood-frequency curve, the maximum discharge in a stream or river each year, or all discharges greater than a certain level (e.g., one that will flood a certain area, like a lowland pasture or structure, like a levee) irrespective of year, are used. Most commonly, the annual maximum instantaneous discharge is used. Gaging records of long duration produce the best flood-frequency analyses. In most cases, at least 20 years of record should be utilized to obtain reasonable predictions. Peak discharges are listed according to magnitude with the highest discharge first. Probability of exceedance, P, is calculated as:

$$P = \left[\frac{m^*}{(n^*+1)} \right] 100\%$$
(3.11)

The recurrence interval, T (usually in years), is calculated as:

$$T = (n^* + 1)/m^* \qquad (3.12)$$

where n^* is the number of years of record and m^* the magnitude of the flood by its rank ($m^* = 1$ at the highest discharge on record). Each flood discharge (y-axis) is plotted against it probability of exceedance or recurrence interval on probability paper. The points are joined to form a flood-frequency curve or exceedance curve (see Figures 3.4 and 3.5). Even without extremely long-term records, the curves are often projected to calculate discharge for a 100-year event. In turn, that 100-year event discharge can then be compared to a rating curve for the stage-discharge relationship and an estimated height required for a levee or building to withstand that even can be estimated. The stage-height of 100- and 200-year events are frequent structural design criteria as well as designated floodplain boundaries for flood insurance determination (Linsley *et al.* 1992, Cech 2003).

III. SPECIFIC METHODS

A. Basic Method 1: Volumetric Analysis

This is the most accurate technique but can be used only in places where the flow is concentrated—for example, the V-notch of a permanent weir or the outflow of a pipe or culvert under a bridge or highway. Thus, this approach works well only for the lowest discharge conditions or for low-order streams.

1. Choose a container of known volume or graduated with known volumes. It should be of at least 4 liters capacity (for stream orders greater than 2 or 3, a larger volume may be required). As an alternative, you can use a heavy-gage plastic garbage bag that can be held down and open on the streambed.
2. Place the container under the outflow and begin recording the time it takes to fill the container to the known volume mark. If you are using the plastic bag, simply record the time necessary to fill a portion of the bag (you will then have to pour out the bag into a measuring container to obtain the volume collected). A stopwatch is best for the timing and should be started at the exact time the container is placed into the flow. Be sure that the volume of the container is sufficiently large that it takes at least three seconds or longer to fill the container. A more accurate measurement would be to start the timing as the level passes a certain graduation and stop it when the level passes yet another.
    Discharge is calculated as:

$$Q = \forall /t \qquad (3.13)$$

where Q is the discharge in m³/s (or liters/s); ∀, volume in m³ (or liters); and t, time (s).

3. Several readings should be taken to obtain a mean and variance in the measure.

B. Basic Method 2: Velocity-Area Method

1. Stretch a measuring tape across the stream and divide it into at least 10 intervals or cells.
2. Alternatively, a premarked tagline can be used; however, you may have to make marks between the permanent marks in order to get 10 or more intervals. In any case, no individual interval should exceed 3 m.
3. Record the width (m) of each cell.
4. At the interval point of each cell, measure the depth (m) and record (see Figure 3.8).

Float Protocol for Estimating Velocity

1. Measure a length of stream equal to at least 20 m to assure a travel time of at least 20 seconds. This is the designated reach length, L. This section should overlap one of the sections being measured for cross-sectional area. Mark the upper and lower ends of this interval with a stake (or stretch a light string across the stream).
2. Choose a float that in only slightly buoyant. This will allow the object to move smoothly with the main vector of flow and minimize the influence of air currents. An orange (peeled oranges float lower in the water), a chunk of ice, a half-filled fishing float or bobber (or tennis ball), or waterlogged branch is ideal.
3. Introduce the float a slight distance upstream of the upstream mark so that the float can reach the speed of the water before it passes the first mark. In large rivers (>10 m width), divide the stream into thirds and make several passes with the float in each third to obtain an average velocity.
4. Use a stopwatch to measure the time (t) of travel of the float between the upstream and downstream marks. Record several measurements through each section to obtain an average. Surface velocity (V_s) is calculated as:

$$V_s = L/t \qquad (3.14)$$

5. A correction factor, k, for the roughness of the bed that affects the slope of the velocity profile must be applied to get an estimate of the mean velocity, V:

$$V = kV_s \qquad (3.15)$$

6. The correction factor varies between 0.8 for rough beds to 0.9 for smooth beds, but 0.85 is most commonly used unless a singularly rough or smooth bed is being measured.

7.   Calculate discharge as:

$$Q = V \left[\sum (A_i) \right] \qquad (3.16)$$

where A_i is the cross-sectional area of each cell or interval along i intervals.

Current Meter Protocol for Estimating Velocity

1.   At each interval point (vertical), place the current meter into the stream with the meter facing into the current. Be certain to stand downstream of the measuring device. Make sure that eddies around legs do not disturb the activity of the current meter.
2.   If depth (D) is less than 60 cm, read the velocity at $0.4 \times D$, measured upward from the streambed. If depth is greater than 60 cm, read and record velocities at $0.2 \times D$ and $0.8 \times D$. The mean velocity is the average of the two readings.
3.   If the water column for the cell being measured contains large submerged objects (logs, boulders, etc.) or is disturbed by overhanging vegetation, read and record velocities at $0.2 \times D$, $0.4 \times D$, and $0.8 \times D$.
4.   Calculate mean velocity as:

$$V = 0.25(V_{0.2} + V_{0.8} + 2V_{0.4}) \qquad (3.17)$$

5.   If velocities are extremely high or flood flows exist and it is difficult to place the current meter and wading rod (or sounding cable) into the water and maintain a vertical position, measure and record the velocity at the surface.
6.   Calculate mean velocity using Eq. (3.15), where k is usually 0.85.
7.   Calculate and record discharge for each interval (x) as in Eq. (3.6).
8.   Discharge for the transect is calculated as the sum:

$$Q = \sum_{0}^{n} q_x \qquad (3.18)$$

when n intervals have been measured from the datum.

C. Advanced Method 1: Slope-Area Method

This is an indirect method for estimating discharge when no gaging information is available. Most often this is used to estimate discharges at high flows such as bankfull

flows or recent flood events. It can also be used when a current meter or float is not practical (e.g., low flows that barely cover the stream bed). However, it should be noted that the accurate assessment of *Manning's "n"* is more difficult.

1. Choose a straight reach of stream where flows are reasonably uniform. The water slope and channel bed slope should be relatively parallel. The length of the study reach should be at least six times the mean channel width (the average recurrence interval of pools and riffles). The important factor is that a pool and riffle pair is included for the best estimate of average slope.

2. Stretch a measuring tape across the stream and divide it into at least five cells. In any case, no cell should be wider than three meters. Measure and record the width of each cell.

3. At the center point of each cell, measure and record the depth (m).

4. Identify the water level of interest. This does not necessarily have to be the present water surface elevation. Levels such as bankfull or high water marks (i.e., indicating the last flood) can be flagged with surveyor's tape or markers.

5. Surveys should be made for three or more typical cross sections in the reach. At each survey point, set up a surveyor's level, able to swivel to see points at least 20 m upstream and downstream of the transect. In some instances, this may be a clear position along the bank or a position on a midchannel bar.

6. Using a surveyor's level and leveling rod, measure and record bed elevations and water surface elevations at points 20 m upstream and downstream of the transect (without moving or repositioning the level). For bed elevations, the rod should be placed at or near a point equal to the average depth and close to the *thalweg*. For water surface elevations, the rod holder should just touch the surface of the water several times while elevations are recorded. The average of three or four of these readings will be acceptable as water surface elevations.

7. During a walking survey of the stream reach, estimate *Manning's "n"* according to values printed in Table 3.1. or Table 3.2. Strickler's (1923) estimate of a minimum value for deeper channels where depth of flow is at least three times greater than

TABLE 3.2	Typical *Manning's "n"* Values for Low-Order, Natural Streams (bankfull stage <30 m) [Adapted from Chow (1959)].	
Channel Condition		*n*
Lowland and Foothill Streams		
Clean, straight, no deep pools		0.030
Clean, straight, some cobble and weeds		0.035
Clean, winding, some pools and riffles		0.040
Clean, winding, pools, riffles, some cobble and weeds		0.045
Clean, winding, pools, riffles, many cobbles		0.050
Sluggish, deep, weedy pools		0.070
Weedy reach, deep pools, riparian with stands of timber and brush		0.100
Mountain Streams		
Streambed of gravel cobble, and a few boulders		0.040
Bed of medium and large cobble and boulders		0.050

the median diameter (D_{50}) of streambed material projecting into the flow is
calculated as:

$$n = 0.04 D_{50}^{1/6} \tag{3.19}$$

8. Calculate the cross-sectional area of each cell (A_n) as the product of cell width
    (w_n) and cell depth (D_n). Total cross-sectional area (A) is calculated as:

$$A = \sum A_n = A_1 + A_2 + \cdots + A_n \tag{3.20}$$

9. Calculate the mean depth as an average of the cell depths. For a wide shallow
    stream this approximate value may be used as the hydraulic radius, R; however, at
    bankfull and flood stages, the calculated hydraulic radius should be used. In
    uniform channels the energy slope of the stream (S) is estimated as the difference
    in water surface elevation $(E$, in meters) between the upstream point $(E_{upstream})$
    and the downstream point $(E_{downstream})$ divided by the distance between the points
    $(l$, in meters):

$$S = \frac{\left(E_{upstream} - E_{downstream}\right)}{L} \tag{3.21}$$

Note that, except for the purposes of examining local hydraulic conditions, bed
slope is not appropriate as a substitute for power slope.
10. Calculate discharge for that transect and water surface elevation using Eq. (3.10).

D. Advanced Method 2: Stage-Discharge Method

This method requires many discharge measurements at a number of different water
surface elevations. It is used to construct a gaging system (i.e., a rating curve) for a
particular sampling site that will be visited frequently and a rapid measure of discharge
is required for each sampling visit.

1. Discharge measurements must be made for at least three different water surface
    elevations: low flow, median flow, and high flow.
2. The section that is measured should be accessible at all water surface elevations and
    discharges to be measured. Choose a straight reach of stream where flows are
    relatively uniform. The water slope and channel bed slope should be reasonably
    parallel.

3. At each flow, measure the discharge by the current meter method (or the volumetric method) listed previously.
4. Plot the dependent variable (i.e., discharge) on the *x*-axis and the independent variable (i.e., water surface elevation or stage) on the *y*-axis. The points are plotted on log-log graph paper. In most cases, this will plot the points as a straight line.
5. For most ecological studies, an approximation of the rating curve can be made by visually constructing a straight line through the points that were measured and plotted. In most instances, the line can be safely extended to a discharge 2.5 times higher than the highest discharge measured and 0.4 times the lowest discharge measured (Bovee and Milhous 1978).
6. For a more accurate rating curve, the three flows can be fit to the equation:

$$Q = a(h - z)^b \tag{3.22}$$

    where *h* represents gage height or water surface elevation; *z*, gage height at "zero flow"; and *a* and *b* are regression coefficients.
7. The equation is fitted through simple regression techniques (Graybill and Iyer 1994, or any other standard text on statistics) with the easy availability of graphics and regression software packages such as *Excel©* or *SigmaPlot©*. The regression equation is fitted with $(h - z)$ as the independent variable and Q as the dependent variable, despite the fact that the rating curve was plotted with the axes reversed. The value *z* must be derived by trial and error. The true value of *z* is assumed to be a value that lies on a straight line through the 3 gaged values on the log-log paper. Thus, it is possible to visually estimate *z* by graphical extrapolation and "test" this value into the regression equation (most software packages also provide the ability to extend the regression through the axes to estimate this value, as well). If the *z* value is too small, the plotted equation will be concave downward. If the *z* value is too large, the plotted equation will be concave upward.
8. At the sampling site, place a staff gage into the stream. The staff gage consists of a rod (reinforcing bar, *"rebar,"* of 2.5 cm diameter works well) that has been painted a bright color for visibility and marked at appropriate intervals to match the rating curve. For example, marking can be very 0.1 m and a meter stick used to measure exact distances between major marks to get exact water surface elevations. Commercial staff gages are also available from a number of manufacturers. The staff gage should be placed well enough away from the bank so that the water surface will still wet the gage at the lowest flows. The staff/rod should extend at least one meter below the marked section. Pound the rod into the substratum until the water surface covers the mark at the appropriate elevation for the discharge on the day that the gage is installed.
9. During subsequent sampling trips, read the water surface elevation from the staff gage, compare to the derived rating curve, and record the corresponding discharge for the activities undertaken on that day.

E. Advanced Method 3: Analysis of Flood Frequency, Flow-Duration, and Discharge-Mass Flood-Frequency Protocol

1. Obtain a gaging record for the stream or river to be analyzed. Under optimum conditions, at least 20 years of record should be available. Accuracy is improved with additional years of record beyond 20. Monthly or annual data are adequate for this analysis.
2. List annual peak discharges according to magnitude with the highest discharge, first.
3. The recurrence interval (T) is calculated using Eq. (3.12). As an alternative, a less biased estimate of peak floods (Cunnane 1978) can be produced by calculating the recurrence interval as:

$$T = \frac{[(n^* + 1) - 0.8]}{m^* - 0.4} \qquad (3.23)$$

4. The probability (P) that a given discharge will be exceeded (i.e., probability of exceedance) is calculated using Eq. (3.11), or as the reciprocal of T.
5. Each flood discharge (y-axis) is plotted against its recurrence interval or probability of exceedance on log-probability paper. In theory, the largest flood should plot at $P = 0$, as it will never be exceeded and the smallest at $P = 1$, since it will always be exceeded. In all situations, all of the values obtained from the calculations will plot between these two values because the numerator or denominator has been adjusted to be greater than the number of observations.
6. The points are joined to form the flood-frequency curve. In general, a curve fit by eye can be used if the intention is to provide information on floods with a recurrence interval of less than $n^*/5$. Of course, available computer software makes fitting lines to these data easier. When eye-fitting the straight line, greater emphasis should be placed on the middle and high discharge events since the primary purpose is to estimate the height of flood events. For recurrence intervals greater than $n^*/5$, where greater accuracy is required, a theoretical probability should be fit to the data to obtain more reasonable estimates. The standard method applied by the USGS is the *log Pearson Type III* distribution (Haan 1977).

Flow-Duration Protocol

1. Obtain a gaging record for the stream or river to be analyzed. Under optimum conditions, several years of record should be available. If annual duration curves are the objective, then at least 20 years of record are advisable. However, daily, weekly, or monthly data can also be used to examine flow duration over shorter intervals.
2. All flows during the given period (daily, monthly, yearly, etc.) are listed according to their magnitudes.
3. The range of discharges should be partitioned into 20 to 30 intervals. For example, if the total range of discharges for daily records was from 10 to 300 cms, the researcher might enter the intervals as 0–10, 11–20, 21–30, . . ., 291–300.
4. The percentage of time that each interval was equaled or exceeded is then calculated and plotted on a semilogarithmic plot; putting percentages on an arithmetic scale on the x-axis and the log of the discharge on the y-axis.

5. A manual of duration curve interpretations has been published by Searcy (1959) and can be used to analyze specific situations in which dilutions for pollution or catchment storage/flow durations for irrigation, hydropower, or transport of particulates or sediment is necessary.

6. Another valuable application of flow duration curves is to break the total record into 20-year increments and plot separate duration curves on the same graph. The changes in slope and shape are indicators of the impact of land-use change on the storage and dilution capabilities of the catchment.

Discharge-Mass Protocol

1. Obtain the gaging record for the stream or river to be analyzed (see USGS website for access). Under optimum conditions, several years of record should be available. If annual duration curves are the objective, then at least 20 years of record are advisable. However, daily, weekly, or monthly data can also be used to examine discharge-mass relationships over shorter intervals of time. Traditionally, monthly total discharge values are used.

2. Cumulative discharge values for each month are plotted against the time intervals involved (see Figure 3.6).

3. A flow rate index based upon critical discharge values (e.g., discharges required for incubation of eggs, spawning, instar success, or year-class strength) are compared to the slopes of the mass curve to determine the percentage of time, historically, a certain flow rate has been sustained.

4. Newbury and Gaboury (1993) have described various biological applications of mass curve analysis and Chow (1964) has provided information on the use of mass curves for setting flows for reservoir design. Newbury and Gaboury suggest that mass curves can be used to establish minimum flows and indicate the amount of time necessary to recharge a system if those flows are not met or exceeded. These curves can also be used to estimate flows at ungaged sites and to estimate bankfull conditions.

IV. QUESTIONS

1. Consider each of the techniques for directly measuring discharge. Where is error introduced into the calculations?

2. When choosing a sample transect for discharge calculations, what precautions must be taken in order to ensure that the best estimates of mean depth and velocity are obtained?

3. What are the difficulties that can be encountered when attempting to describe the resistance of the channel to flow (i.e., Manning's "n")?

4. In what ways can environmental scientists and engineers use flood-frequency and stage-discharge relationships to design levees and dams yet continue to promote ecological integrity?

5. What is the value of flow-duration curves to the management and analysis of floodplains?

6. What changes in flow-duration curves, flood-frequency curves, and stage-discharge relationships might be expected with changes in land use in a

catchment? That is, how will the curves change if the amount of impermeable surface or bare soil increases over time?

7. How might weeks or days of flow persistence during a sensitive spawning or incubation period be estimated using a discharge-mass curve?

8. For a measured discharge, how much variation was there in the mean velocity between riffle and pool transects? How does this affect discharge estimates?

9. Compare the hydraulic radius and the mean depth for the sample reach at high and low discharges. How would these two values alter discharge predictions?

10. Examine your estimate of roughness, Manning's *n*. What were the major factors that influenced it at the discharge you analyzed? What values will dominate the estimate of Manning's *n* at higher or lower flows?

V. MATERIALS AND SUPPLIES

Discharge Measurements

> 4-liter or larger bucket or wide-mouthed container
> Calculator
> Current meter with wading rod (any of the standard meters: Pygmy, Price Type AA, Ott, Marsh-McBirney)
> Float
> Meter Stick
> Reinforcing bar (*rebar*) (2.4 cm diameter) — 2 to 3 m length
> Stopwatch
> Surveyor's level, tripod, and stadium/rod or leveling rod
> Tape measure (at least 50 m)

Hydrographs

> Calculator
> Gaging records for local streams and rivers (in the United States, these can be obtained in Government Documents sections of most major university libraries or from area/regional offices of the USGS or directly downloaded from the USGS website at http://waterdata.usgs.gov/nwis/sw. Often, local utility companies will have gaging records from streams near hydropower facilities and will publish these data on line, as well.
> Log-log graph paper
> Log-probability graph paper
> Semilogarithimic graph paper
> (Note: If you use graphing/statistical computer software packages, most of these axis options can be created prior to printing.)

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Dynamics of Flow

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I. INTRODUCTION

A. Flow Forms

Aquatic habitats within and on the boundaries of flowing water possess unique hydraulic characteristics, varying from slowly flowing layers next to the channel boundaries to rapidly circulating turbulent eddies within the flow that are riverwide in scale. Organisms that live in this dense fluid are close to being neutrally buoyant and must have elaborate strategies for holding their position, gathering food, and moving with and against the flow. It would be as if we were hot air balloons, living in neutral density on the surface of the earth and subject to the whims of wind and air pressure in and around trees and buildings.

Although fluid dynamics have been intensively studied over the past two centuries, the complexities of the hydraulics in flowing water has not been fully analyzed. Traditional methods used by engineers to design open channels are empirical, solved primarily by ignoring boundary layers, turbulent eddies, and the parceling of portions of the flow that move up, down, backward, and forward in a natural channel. This complexity of the flow is related to the natural sine and cosine waves in which it moves. Peaks in the waves form riffles, troughs form pools and meanders mimic their horizontal amplitude, creating a broad range of hydraulically unique habitats.

In the branching network of river channels, the habitats are nested within one another at smaller and smaller scales (Figure 4.1). At the catchment scale (Figure 4.1, Level I), the hydraulic condition of the flow may be generalized as uniform or gradually varying above and below interruptions in the longitudinal (long) stream profile (Chow 1959). Uniform flow conditions occur when the slope of the water surface and the channel bed are approximately parallel with little change in the cross-sectional area of the flow. Chezy or Manning type uniform-flow equations can be applied to estimate velocity by
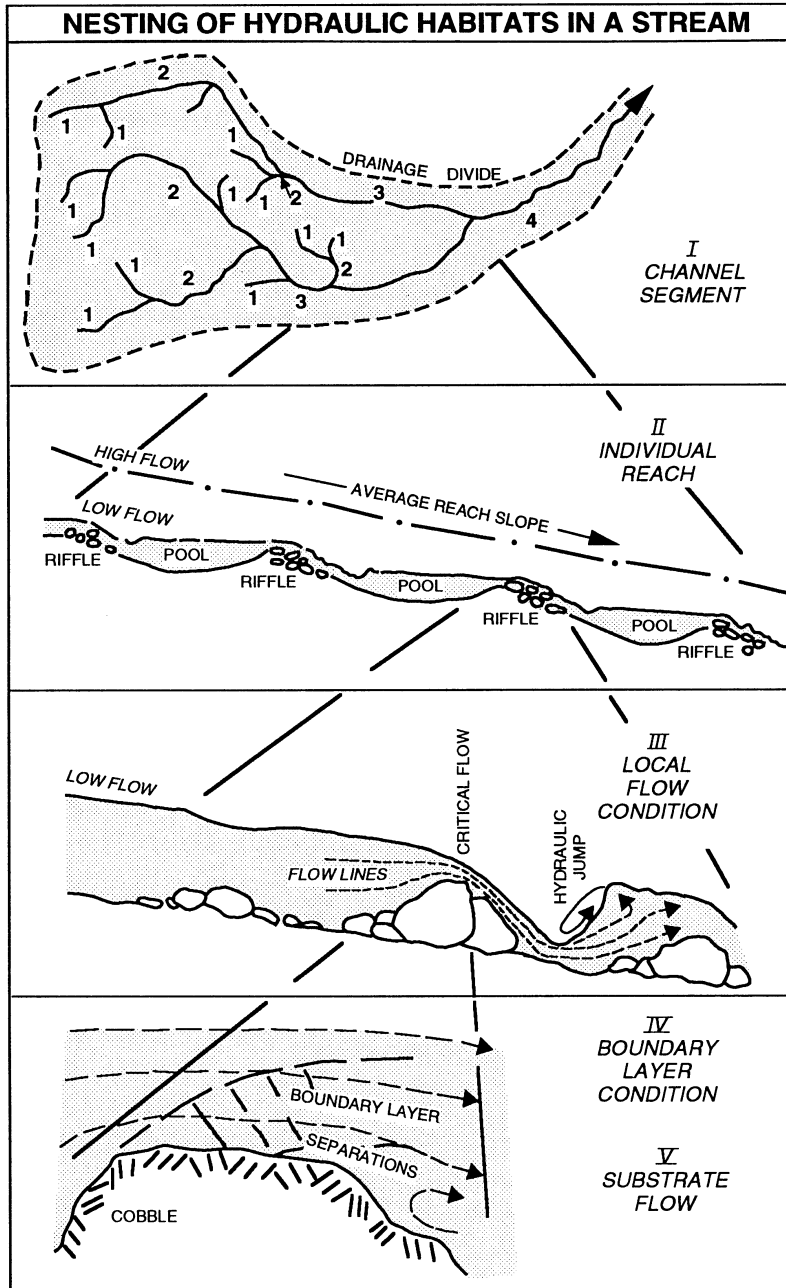
NESTING OF HYDRAULIC HABITATS IN A STREAM

FIGURE 4.1 Hydraulic conditions in a stream viewed from different scales.

assuming frictional resistance on the stream bed accounts for all of the energy losses in the flow (see Chapter 3). In many natural streams, particularly at lower stages, the uniform flow equation must be applied with surrogate resistance factors to account for major obstructions on the stream bed and the turbulent energy losses created by local nonuniform flow conditions (Millar 1999). The uniform flow assumptions, with correction factors for natural channels and floodplains, are an integral part of the runoff and flow models used for instream flow simulation (Milhous *et al.* 1989) and flood routing (Bedient and Huber 1992). The reduction in flow complexity necessary for their solution is a drawback in predicting instream habitat availability (Kondolf *et al.* 2000).

At the stream reach scale (Figure 4.1, Level II), mesohabitats of nonuniform flows can be distinguished. In mobile bed streams the natural sine wave followed by flood flows creates pools and riffles spaced at 2π or approximately 6 times the width of the channel (Leopold *et al.* 1964, Chang 1988, Gregory *et al.* 1994). In many combinations of discharge, slope, and erodible bed material the channel meanders with an average wavelength of 4π or approximately 12 times the bankfull width, forming two pool and riffle reaches in each full meander (Figure 4.2). Channel and meander geometry for midlatitude streams in North America has been summarized by Leopold *et al.* (1964) and Dunne and Leopold (1978).

To distinguish the pattern of nonuniform flows, the mean depth, velocity, and direction of flow may be mapped on sketches or surveyed plans of a reach. Channel configuration and flow conditions are major components used to characterize the preferred habitats of fish, such as the preferred trout meanders described by Newbury and Gaboury (1993) and habitat suitability curves derived for macroinvertebrates and fish (Bovee and Cochnauer 1986, Gore *et al.* 2001, Beecher *et al.* 2002, Mäki-Petäys *et al.* 2002).

At the habitat scale (Figure 4.1, Level III), individual streamflow lines and states of flow can be delineated and partially analyzed with rapidly varied, nonuniform flow equations. The local velocity and depth of the flow is dominated by its momentum and gravity rather than boundary friction (Chow 1959). In riffles or rapids, the flow is broken into segments by cobble bars and boulders that create zones of smoothly accelerating flow
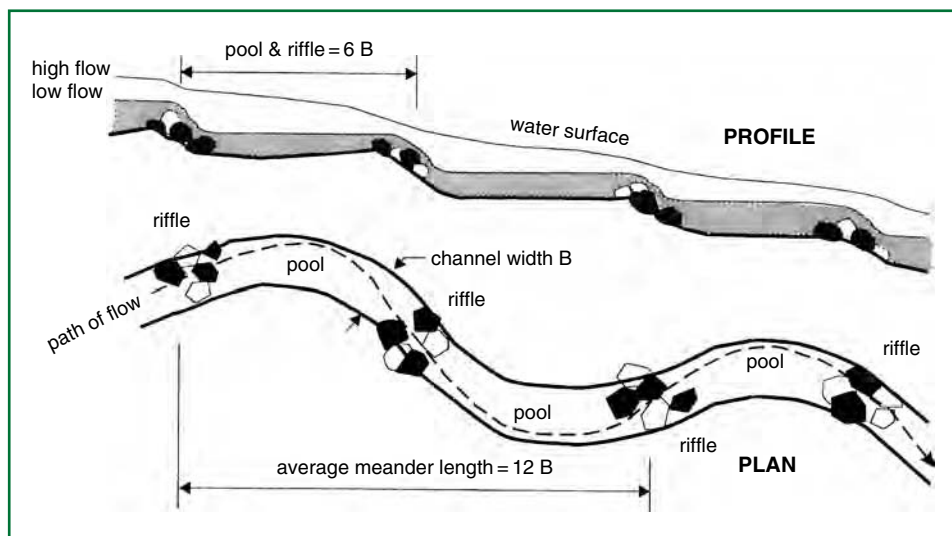

FIGURE 4.2 Average meander, pool, and riffle dimensions expressed as a ratio to the bankfull width.
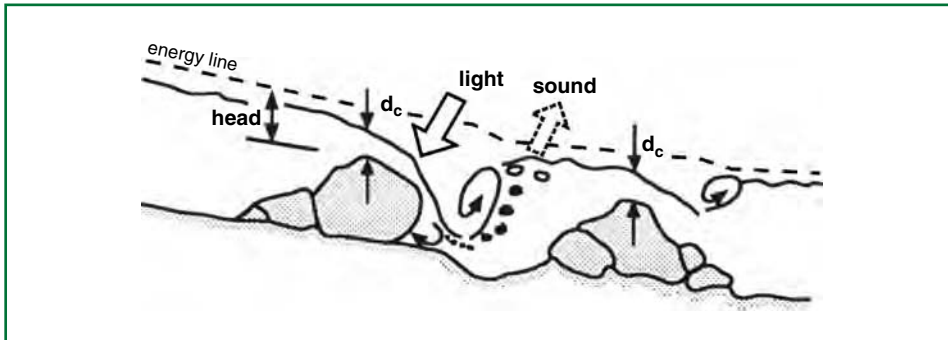
FIGURE 4.3 Rapidly varied flow conditions created by large cobbles and boulders relative to the stream depth. The critical depth at the point of overflow is two-thirds of the upstream head H.

over and around obstacles followed by turbulent areas of deceleration and side eddies. If the flow drops over an obstacle or is drawn through a narrow gap between boulders, it often reaches the critical state, a condition where the velocity is maximized for the head of water that exists above the obstruction (Figure 4.3). The critical depth of flow occurs as the water passes over the obstruction and is equal to two-thirds of the upstream head. At the point of overflow, the critical velocity (V_c) can be computed as a function of the depth:

$$V_c = (gd_c)^{1/2} \tag{4.1}$$

where V_c = critical velocity (m/s), g = gravitational acceleration (9.81 m/s), and d_c = critical depth of flow (m). This is also the velocity of a wave of disturbance in a still pond at the same depth. Consequently, smooth standing waves of disturbance from the bed slowly shift upstream and downstream in the critical flow zone as the downstream velocity and the disturbance wave velocity upstream are equal. If the water continues to accelerate past the obstruction or gap, it attains supercritical velocity. The accelerating flow is drawn downward in tension, forming a smooth shallow sheet of water—for example, on the downstream face of a submerged cobble. Maximum light penetration to the streambed occurs in these short segments. This thin, clear window to the bed enables algal growth to flourish. The supercritical condition terminates abruptly when the flow shifts tumultuously back to subcritical conditions as it enters a local pocket or pool of slower-moving water downstream. The supercritical flow penetrates the slower-moving pool, forming a surface back-eddy entrained with air as it climbs to the greater downstream depth in a hydraulic jump. Light penetration through the air entrained section is dramatically reduced, and it may form refugia from predators for smaller fish. This is also the source of noise in the river. Uniform flow makes no noise regardless of its velocity, but the breaking bubbles of the air carried into the flow makes brooks babble and rapids roar. In some cases it is a significant attraction beacon for migrating fish below dams.

The state of flow relative to the critical velocity is characterized by the Froude number *Fr* (Henderson 1966):

$$Fr = V_m/(gd)^{1/2} \tag{4.2}$$

where V_m = mean velocity of the flow (*m/s*) and d = depth of flow (*m*). Values equal to 1 represent critical flow, <1 subcritical flow, and >1 supercritical flow.

Critical and near-critical flows are efficient habitats for net-spinning caddisflies, black flies, and other benthic insects. By locating on the tops and sides of boulders in this zone of converging flow, they are able to expand their capture nets or cephalic fans to efficiently gather detritus as it exits a pool or run segment (Wetmore *et al.* 1990; also see Chapters 12, 20, and 25). Nonuniform local flow patterns also create protected habitats and feeding opportunities for fish. At high flows, when pools are subject to scouring velocities, fish move into calmer water zones created in eddies behind boulders, debris, and other irregularities. These diverse flow conditions allow fish to navigate rapids in short bursts between protected water pockets or by launching themselves over obstacles from the upstream surface current below a hydraulic jump (Stuart 1962).

The turbulent structure of the flow within a hydraulic habitat is more difficult to analyze. Reynolds (1842–1916) proposed a dimensionless number that would describe the scale of turbulence relative to a characteristic body length or depth. The Reynolds number Re is the ratio of the inertial forces of the moving fluid to the viscose properties of the fluid that resist mixing. This is characterized by the ratio of the velocity of the flow times a characteristic length at the scale of interest—for example, an organism body size or depth of flow—to the kinematic viscosity:

$$Re = VD/\eta \tag{4.3}$$

where V and D are in *m/s* and *m*. Typical values of η range from $1.8 \times 10^{-6}\,\text{m}^2/\text{sec}$ for water approaching 0°C, 1.3×10^{-6} at 10°C and $1 \times 10^{-6}\,\text{m}^2/\text{sec}$ at 20°C. The choice of a characteristic length depends on the size of the phenomenon under study. The depth of flow may be used in studies of large eddies, but the length of a fish or insect measured in the direction of the flow may be used in studies of forces that act directly on the organism. When the Re numbers fall below 2000, the viscosity of the water dampens turbulence and the flow becomes increasingly laminar. Re numbers are much larger than 2000 in the main body of the flow, but a very thin laminar layer may exist next to the channel boundary. The laminar boundary layer in a 1 m deep gravel bed stream flowing at 1.3 m/s would be less than 1 mm thick. The other end of the velocity range where Froude numbers exceed critical values in waterfalls and steep chutes is limited in distribution as well. The ranges of Fr and Re numbers observed in natural streamflow from the boundary layer surrounding fixed objects to supercritical rapids and waterfalls fall within the envelope of depths and velocities shown in Figure 4.4.
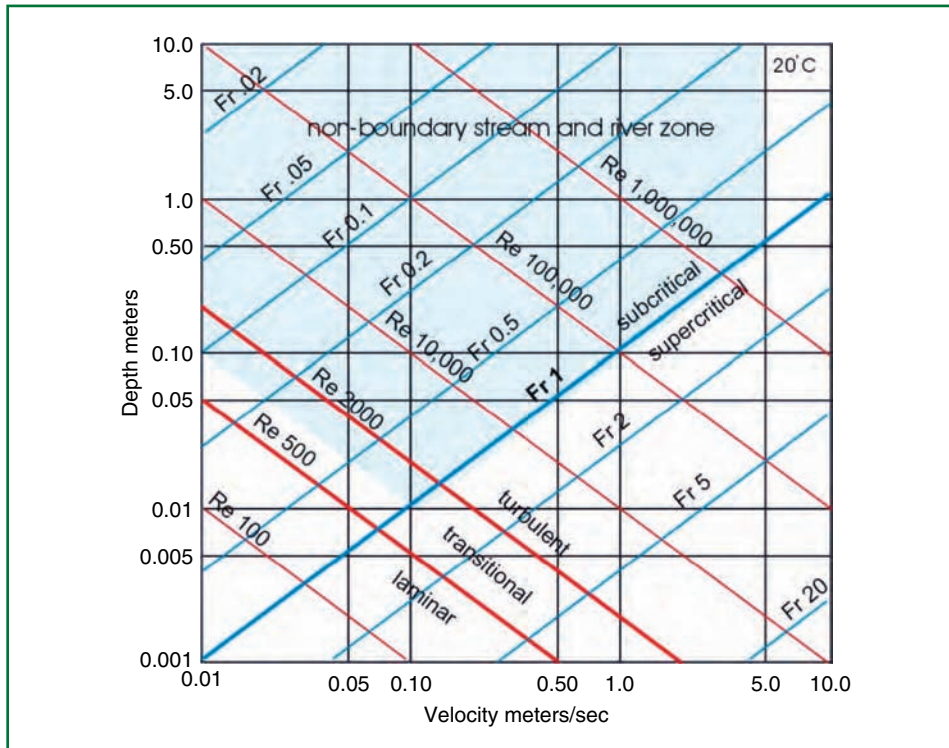
FIGURE 4.4 Velocities, depths, Froude numbers (Fr), and Reynolds numbers (Re) encountered beyond the boundary layer in rivers and streams generally lie below Fr = 1 and above Re = 2000. Fr numbers >1 occur locally in overflowing chutes and waterfalls.

Preferred habitats with distinct ranges of Fr and Re have been found in various studies (Bisson *et al.* 1988, Heede and Rinne 1991). For example, the hydraulics of preferred locations mapped for wild and hatchery one-year-old cutthroat trout in British Columbia streams showed that tank-raised hatchery fish conditioned by mechanical circulation systems spread into areas of flow seldom used by the wild fish. Their preferences overlapped with areas inhabited by large predators that were subject to the main torrent of flood flows (Figure 4.5). The flushing of juvenile hatchery fish from the sample reaches after one or two flood flows was almost complete (Bates 2000).

Fr, Re, and the local flow pattern may be mapped by measuring velocities and depths in the pools, riffles, and back eddies of a typical stream reach (Panfil and Jacobson 1999). For example, where the trajectory of high velocity flows enters slower moving water at the end of a riffle or contraction in the channel, the flow separates into several characteristic cells (Figure 4.6). The largest cells in return eddies are driven by the shear force of the rapid flow as it penetrates the slower flow. The higher velocities and consequently larger kinetic energy component of the rapid flow decreases the water elevation. This causes a shallow gradient and a velocity component toward the center of the channel. Consequently, the cell is inclined along the lateral gradient with upwelling flow on the outside leg and downwelling flow on the inside leg under and adjacent to the rapid flow trajectory. Where a high-flow torrent is split by a midstream obstacle, four vortices are formed: two on the sides of the channel and two smaller vortices immediately below the obstacle.
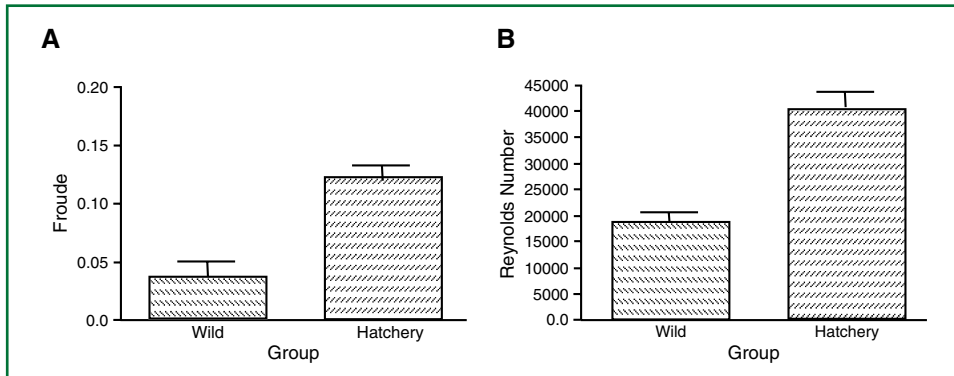
FIGURE 4.5 Froude number (A) and Reynolds number (B) preferences in a natural stream made by wild and hatchery-raised one-year-old cutthroat trout (Bates 2000). The higher Froude number areas occur in the deep central channel that is scoured by floods. The lower Froude number areas occur in shallow back eddies on the margins of the channel that shift toward and ultimately onto the floodplain as the discharge increases.
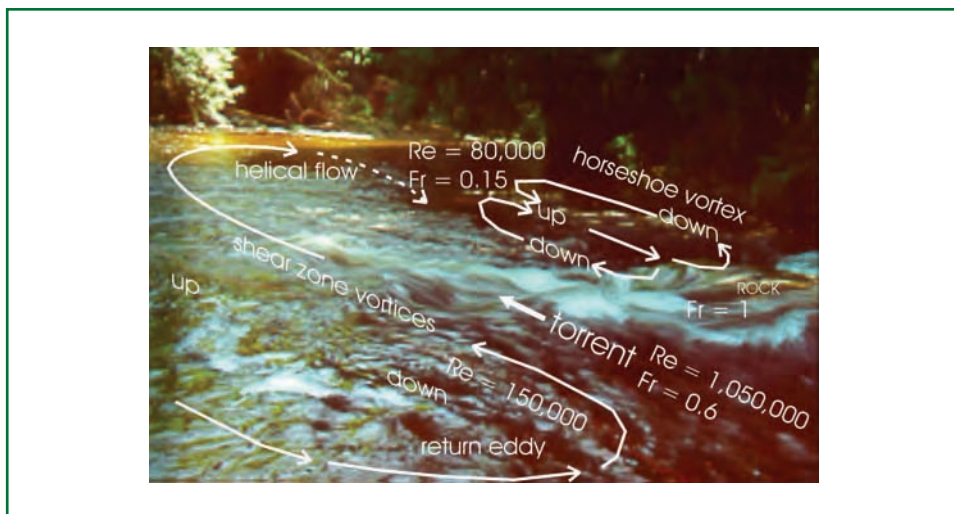
FIGURE 4.6 The trajectory of rapid flow (Re = 1,050,000) entering a slower moving pool (Re = 80,000) causes flow separations and back-eddies to form. The large rock splits the flow, forming a double back-eddy or horseshoe vortex downstream. Part of the flow reaches critical velocity as it passes over the rock (Fr = 1) (Chapman Creek BC, discharge = 4 m^3/s).

The latter two vortices combine to form a single upstream flow that is downwelling as it approaches the downstream face of the obstacle (aptly named a horseshoe vortex by kayakers observing the surface pattern). This vortex plays a major role in capturing surface detritus and delivering it to biota on the back and lower boundaries of the obstacle. Upstream, downstream, and stationary positions can be assumed by a drifting body with little expenditure of energy by choosing an appropriate position in the vortices.

At the microhabitat scale (Figure 4.1, Level IV), local flow conditions must be characterized indirectly as there are limited techniques for direct field measurements. This is the scale at which near-boundary laminar flow conditions can be detected. Although the main body of the flow in streams is turbulent, as the fixed boundary of the flow is approached, the velocity decreases until the viscous forces overcome turbulence and the flow becomes laminar. In the laminar boundary layer, the water moves in parallel lines without mixing. There are several useful studies and opinions regarding its importance in this recently developing area of hydraulic stream ecology (Nowell and Jumars 1984, Statzner *et al.* 1988, Carling 1992). Similarly hyporheic flow within the bed materials requires detailed measurements and sampling. The importance of these microhabitats to aquatic organisms and discussion of this near- and subboundary habitat are discussed in detail by Vogel (1994) and Boulton *et al.* (1998).

B. Flow Forces

General Shear Stress

Analysis of the general shear stress or "tractive force" exerted by the flow on the stream bed is based on the same simplifying assumptions that were used in deriving the uniform flow velocity equations. The total energy of the flow at any point in an idealized stream channel can be expressed in units of height above a datum. The three energy components are the elevation of the channel bed, the depth of flow, and the kinetic energy of the flow. The sum of the three components or total energy E is described in Bernoulli's equation:

$$E = bed\ elevation + d + V_m^2/2g \qquad (4.4)$$

The specific energy of the flow is composed of the depth and kinetic energy at that bed elevation. An imaginary line may be drawn through the position of the total energy of the flow that is elevated above the water surface by the amount of the kinetic energy (Figure 4.7). In uniform flow the slope of the energy line is assumed to be nearly parallel to the slope of the bed and water surface. If the slopes are not parallel, the energy line slope is used in flow analyses (Chow 1959). The down-slope component of gravity acting on the water mass parallel to the energy line slope drives the flow downstream. The motion is resisted by friction on the bottom and sides of the channel. Assuming that the mass of water moves as a solid block along the inclined streambed at a constant velocity, the sum of the frictional forces or tractive force T_G is equal to the down-slope gravity component:
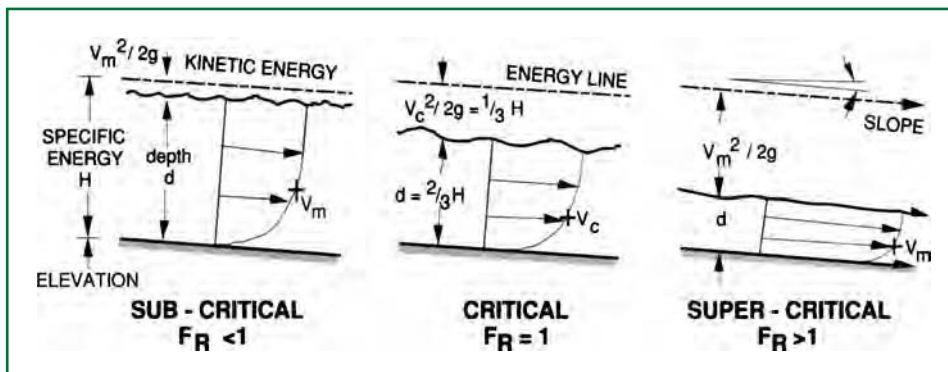
$$T_G = \rho g R S \qquad (4.5)$$

FIGURE 4.7 The depth of flow, kinetic energy, and specific energy line for uniform flow conditions.

where T_G is the tractive force or general bed shear stress (N/m^2), ρ is the density of water ($1000\,kg/m^3$), g is gravitational acceleration (9.81 m/s), R is the hydraulic radius (m) (see Chapter 3), and S is the gradient of the energy line for mildly sloping channels.

Studies of canals and mobile-bed streams have showed that the tractive force is related to the size of material transported and to the substratum stability (Lane 1955). This has been used to describe the effects of substratum instability on the density and composition of benthic insects (Cobb *et al.* 1992).

Local Shear Stress and Boundary Layers

The local shear stress acting on a small portion of the stream bed, such as on the surface of a cobble or on an insect clinging to the cobble, requires a more specific determination. To estimate the local shear stress (τ_0), measurements of velocity at the specific site are required. The relationship between the near-bed shear stress and the velocity profile in the turbulent flows of natural streams is largely empirical with founding laboratory studies conducted by Prandtl, von Karman, and Nickuradse (reported in Chow 1959). The velocity profile above a rough boundary has been characterized in three segments: (1) a laminar flow viscous layer immediately next to the wetted channel boundary, (2) a buffer zone or transition layer of viscous turbulent flow, and, for the major part of the profile, (3) a fully developed turbulent layer in which the velocity is distributed logarithmically with the height above the stream bed (Roberson and Crowe 1993). The velocity profile in the turbulent zone predicted by the Prandtl-von Karman universal velocity distribution law may be written in the form:

$$u/u_f = 5.75(log\ y - log\ y_o) \tag{4.6}$$

where $u =$ velocity at location y in the turbulent layer (m/s), $u_f =$ a reference "friction velocity" defined as $u_f = (\tau_o/\rho)^{1/2} (m/s)$, $y =$ distance above the stream bed (m), $y_o =$ height

at which the logarithmic velocity profile extrapolates to zero (m). The local shear stress at the sampling site is then

$$\tau_o = \rho(u_f)^2 (N/m^2) \qquad (4.7)$$

by definition (Chow 1959).

Using sand grains as roughness elements, Nickuradse demonstrated that $y_o = k/30$, where k was the diameter of the sand grains (Chow 1959). In some stream habitat studies, this observation has been extrapolated and substituted in the velocity distribution law for y_o by assuming that $k =$ the mean diameter of the substratum paving the stream bed (m). If the depth (d) and mean velocity (V_m) are measured and assumed to be ideally at $0.4 \times d$ from the channel bottom (see Chapter 3), the equation may be rearranged to estimate u_f as:

$$u_f = \frac{V_m}{5.75 \left(\frac{log\ 12d}{k} \right)} \qquad (4.8)$$

Alternately an estimate of the local bed shear stress may be obtained by measuring the velocity profile perpendicular to the channel boundary and plotting the relationship $u = f\ (log\ y)$. By fitting a line to the plot, the abscissa y_o at which the logarithmic velocity distribution is nominally zero can be found, allowing the value of u_f to be determined at the measurement site. If the slope of the regression line, $u/(log\ y - log\ y_o)$, is substituted in the velocity distribution law, then u_f is equal to the slope divided by 5.75.

If the flow is transparent and shallow, the shear stress may be characterized with a graded set of shear stress testing hemispheres (FST hemispheres; Statzner and Muller 1989). The shear stress required to move the FST hemispheres was determined by measuring the velocity profile and solving the Prandtl-von Karman formula with the same assumptions as described above (Statzner *et al.* 1991). FST hemispheres have been used to determine the effects of shear stress on benthic insects and to characterize riverine habitats (Peckarsky *et al.* 1990, Gore *et al.* 1994). They have also generated a useful discussion of stream habitat measurements and their interpretation (Carling 1992, Frutiger 1993, Frutiger and Schib 1993, Statzner 1993).

Macroinvertebrates and plants living on submerged surfaces exploit areas requiring minimal energy expenditure. Low velocity habitats exist in the viscous boundary layer $\delta_N (m)$ that has nominal thickness estimated as:

$$\delta_N = 11.8 \left(\frac{\nu}{u_f} \right) \qquad (4.9)$$

where $v=$ the kinematic viscosity of water (m^2/s). This approximation is based on sand grains and should be viewed only as an index of relatively smooth patches on the stream boundary [see "the law of the wall" (Roberson and Crowe 1993) and the discussion of Carling (1992)]. Applying measures that have so many approximations requires some care. One should follow Vogel's sage advice, "don't perpetuate the practice of equation-grabbing predecessors, . . . don't use formulas unless they demonstrably apply, . . . and don't be intimidated by the prospect of measuring low flows in small places" (Vogel 1994).

Hydraulic Forces Exerted Directly by the Flow

An organism or plant that is clinging to the boundaries or resting on the bottom of the channel is subject to the direct force required to block all or a portion of the approaching flow. The force may be approximated as the difference between the upstream and downstream static pressure plus the change in momentum of the flow (Chow 1959), or:

$$F_d = \rho q C(V_{up} - V_{down})/9.81 \tag{4.10}$$

where $F_d=$ the force exerted by the flow (kg), $\rho=$ the density of water at the earth's surface ($1000\,kg/m^2$), $q=$ the blocked discharge that would have flowed through the cross-section of the submerged object perpendicular to the flow (m³/s), and ($V_{up} - V_{down}$) is the net change in velocity approaching and immediately downstream from the body. In the case of an impermeable body, $V_{down}=0$. The value of C, the drag coefficient, depends on the turbulence of the flow and the streamlining of the body. For large, blunt objects, the drag coefficient approaches 1.0. Streamline shapes have much lower coefficients—for example, the value for a sphere is 0.2 and for a trout body 0.015. The force exerted on a blunt impermeable object that is submerged on both the upstream and downstream sides can be approximated by rounding off and reducing equation 4.10 to:

$$F_d = 100\,AV^2 \tag{4.11}$$

where A is the submerged cross-sectional area blocking the flow (m²). For further reading, Vogel (1994) presents an interesting discussion of shapes and drag coefficients and their effect on flow patterns and lateral water pressures.

Substrata and Stream Bed Stability

The stream bed materials (substrata) may be sampled by different methods, depending upon the habitat being investigated. In studies of hyporheic habitats, all sizes of the bed materials are sampled by excavating a portion of the bed or by withdrawing a frozen core from the bed (Platts and Penton 1980, Bunte and Abt 2001). In studying the stability and flow resistance of a reach, the substrata may be sub-sampled for only the largest sizes of

bed materials projecting into the flow. This material is described as the bed paving sample in the sense that it is the largest protective fraction that must resist the bed shear stress (see Chapter 7). Methods of sampling the bed paving materials can be more sophisticated, but for practical bed stability and channel roughness studies a simple random selection "pebble-count" method is adequate (Statzner *et al.* 1988, Wolman 1954). The sizes of bed paving materials may be conveniently summarized in a cumulative frequency plot.

The relationship between the general bed shear stress and the size of particle that can be transported has been widely researched for rip-rap designs in river engineering. A summary of the critical tractive force T_c and the diameter of bed material at incipient motion often reproduced in later works was prepared by Lane (1955) (Figure 4.8). For noncohesive bed materials that are greater than 5 mm in diameter, a simple enveloping relationship for the minimum particle size at the point of incipient motion is:

$$T_c \ (\text{kg/m}^2) = \text{diameter(cm)} \tag{4.12}$$

To convert to Lane's units, $T_c \ (kg/m^2) = T_G \ (N/m^2)/9.81$.

In a uniform channel the mean size of bed material that can be moved at a given discharge may be estimated with Lane's relationship by solving for the tractive force at the corresponding depth of flow. An index of the fraction of the bed paving materials that can be moved at that discharge may be obtained by examining the cumulative frequency plot of the bed paving materials (Newbury 1984). This estimate may be modified for bimodal distribution of stream bed sediments. These general techniques for measuring bed shear stress and instability have been applied successfully in recent studies of the effect of bed instability on the density and species composition of stream insects (Cobb *et al.* 1992) and the shear stress in refugia (Lancaster and Hildrew 1993).

The hydraulics described in this section have focused on identifying flow patterns and habitats in a sample stream reach. Advanced topics deal with flow characteristics within the habitats and the forces exerted by the flow near the stream bed that affect substratum stability and organisms that cling to the stream boundaries. This is an area of active research and discovery and lotic ecologists should be aware that their observations of hydraulically created habitats may significantly contribute to the growing field of knowledge.

II. GENERAL DESIGN

Basic methods classify and map hydraulic phenomena in the whole reach and determine the average conditions of streambed stability at the bankfull stage. Advanced methods quantify the local hydraulic conditions and bed shear stress, assuming that the basic mapping of the sample reach has been completed. The analysis of the data gathered in the field requires plotting, curve fitting, and elementary algebraic calculations. Cross-section, slope, and bed material data may be available in advance from conducting the methods described in Chapters 2 and 3.
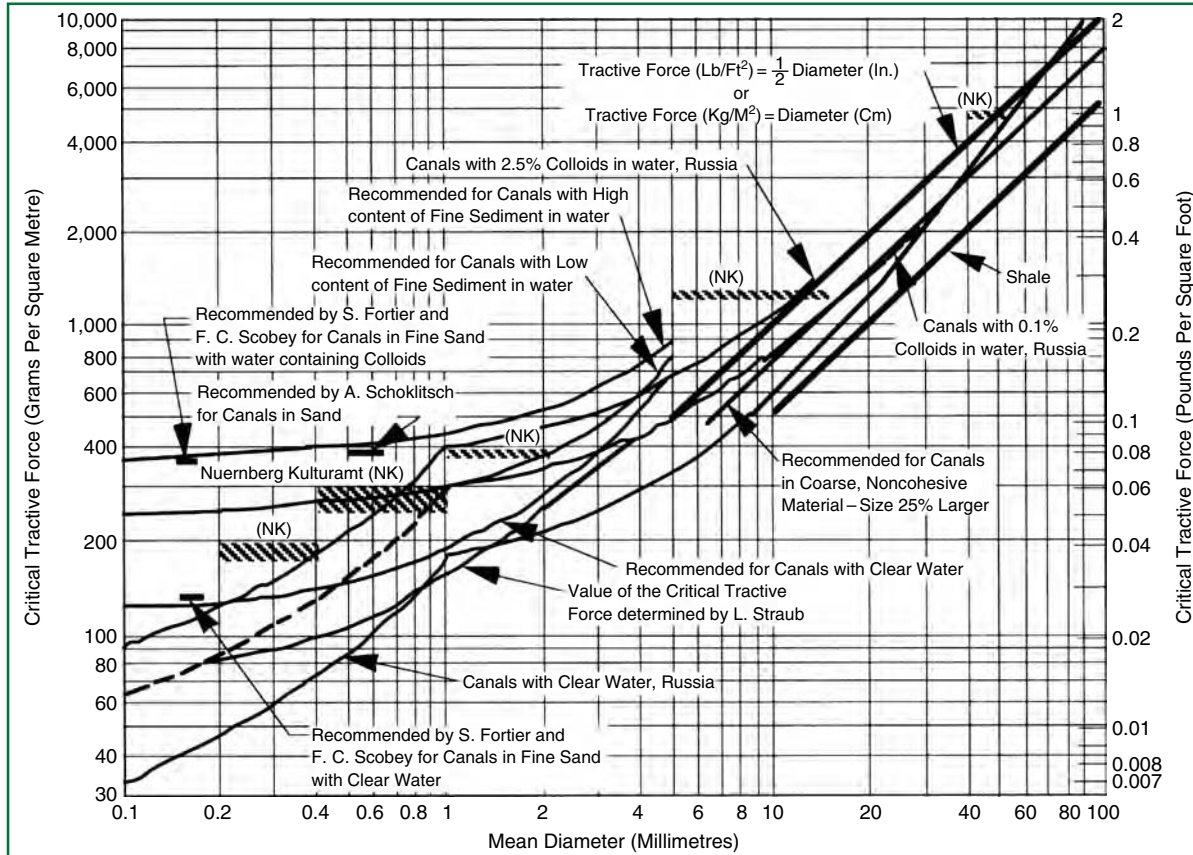
FIGURE 4.8 General relationship between the tractive force and size of bed material at incipient motion prepared from observational data gathered by Lane (1955).

Site Selection

Sample reaches should be selected by examining a topographic plan of the drainage basin at a scale that distinguishes riffles, pools, and meander patterns. Sample reaches should be at least 6 times the bankfull width of the stream and, if present, include at least one full pool and riffle sequence. In smaller meandering streams, a full meander length of two pool and riffle reaches is preferred. The sample reaches may be consecutive in a single branch of the stream or selected in different stream order branches from the top to the bottom of the drainage basin. This will illustrate how the river characteristics and hydraulic conditions change with the drainage area, slope and discharge.

Reaches that do not meet the ideal alluvial form because of bedrock, manmade, or other intrusions or obstructions should not be ignored but treated as special conditions that illustrate deviations from characteristic reaches elsewhere in the basin. Researchers[1] should visit these special reaches to understand their significance relative to their sample reach (e.g., to fish blockage, water diversion, flow regulation, profile control, refugia).

III. SPECIFIC METHODS

Laboratory Preparation for All Methods

1. Identify the drainage basin and stream order segments on a topographic map (see Chapter 2).
2. Plot the long profile of the stream using the contour lines intersecting the stream channel and locate the sample reaches on the profile. Note special reaches that may affect the general hydraulic conditions in the study reaches.
3. Copy the reach data and sketch sheet (Appendix 4.1) or similar equivalent sheets on waterproof paper for use in the field.
4. Test flow meter batteries and survey equipment.

A. Basic Method 1: Mapping Hydraulic Conditions and Habitats

1. Sketch: stretch a tape along a straight baseline on one bank of the reach and sketch in the bottom and top of the channel boundaries and major physical features such as boulders, logs, debris, and typical substrata. Measurements of features in the stream and floodplain may be made with a second tape held perpendicular to the baseline tape. The map should be scaled to include a reach that is at least six times the bankfull width (Figure 4.9).
2. Cross-sections: select four to six typical cross-sections of the channel and measure the average depth and width at the bankfull stage. A tape may be stretched across

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[1] The study reaches should have depths and velocity combinations that can be safely waded. Abt *et al.* (1989) found that the product of the depth (m) and velocity (m/s) before waders were swept away could be as low as 0.7. In larger streams, this may restrict fieldwork to periods of low or moderate flows. Photographs or video recordings of the sample sites at high flows will help to visualize the reach conditions at the bankfull stage. Additional sampling gear such as plastic viewing boxes and snorkeling gear are useful in identifying preferred habitats of target organisms. Biological sampling with nets, traps and electro-fishing may also be undertaken in conjunction with habitat identification.
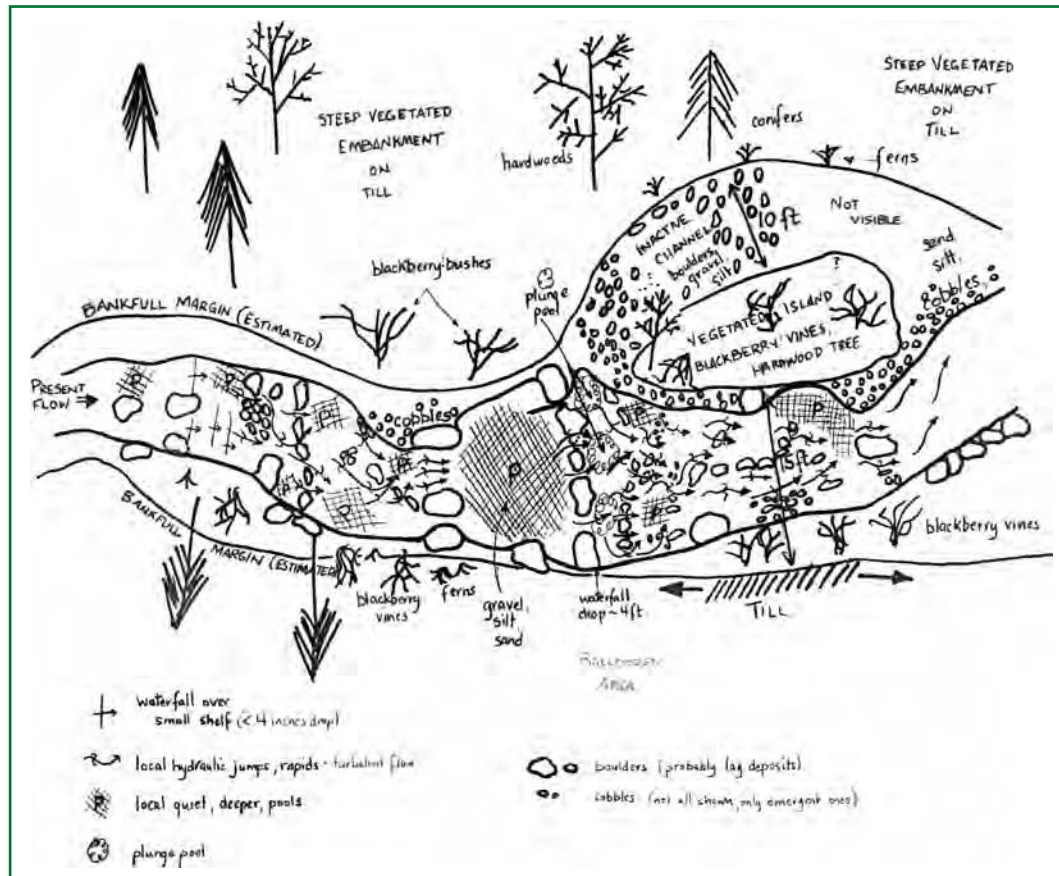
FIGURE 4.9 Sample sketch of a stream reach and features prepared in a student exercise.

the stream at the bankfull stage as a reference for depth measurements. Record the present depth of flow in the sample cross section.

3. Slope: estimate the length and total drop in the reach with a simple level and stadia rod to determine the average slope of the water surface.

4. Locate pools, riffles, and local rapidly varying flow conditions such as hydraulic jumps, chutes, and eddy patterns on the map approximately to scale. Describe and locate any preferred habitat sites that have concentrations of benthic insects or fish. Detailed maps of segments of the reach drawn at a larger scale may be required to show local hydraulic conditions and habitats.

5. Release a float at the top end of the reach and sketch the pattern of the flow by following it along the bank. Repeat the exercise by releasing a float from different positions across the channel cross section and in local zones of hydraulic jumps and back eddies.

6. Redraw the reach map eliminating unnecessary survey lines and measurement notes made in the field.

7. From the sketch and cross sections, determine the average bankfull width and depth. Measure the distance in a straight line between riffles, if they are present, and calculate the ratio of the riffle spacing to the bankfull width.

8. On an overlay of tracing paper, sketch the pattern of flow through the reach at the time of observation and mark in local habitats and flow phenomena. Estimate the area occupied by each flow condition and habitat.

B. Advanced Method 1: Mapping Hydraulic Conditions and Habitats

1. Measure the depth and mean velocity (at 0.4 depth from the bottom) at the uniform and rapidly varying local flow and habitat sites located in Basic Method A. At sites where the critical velocity occurs, measure the depth and velocity in the critical zone. Make a small sketch of the flow configuration noting the area occupied by different flow conditions.

2. Calculate the Froude and Reynolds numbers at each of the velocity and depth sampling sites and plot them on the reach map.

3. Plot the channel geometry of your sample reaches (the bankfull or vegetation trimline widths and depths versus their drainage areas, (Figure 4.10). Compare the slope and intercept of fitted lines to data published for similar regional streams (Dunne and Leopold 1978).

4. Measure the approximate area covered by each of the flow conditions mapped in the reach.

5. Estimate the volume of flow in a critical section from the cross-sectional area and velocity. From the upstream velocity measurements, estimate the cross-sectional area that is contributing flow to the critical section.

C. Basic Method 2: Streambed Stability and Shear Stress

1. Measure the x, y, and z dimensions of a sample of the largest materials that pave the stream bed using a meter stick and record them on the reach survey form (Appendix 4.1). The sample should be randomly selected by wading through the reach and selecting the largest sizes projecting from the bed surface every few steps.
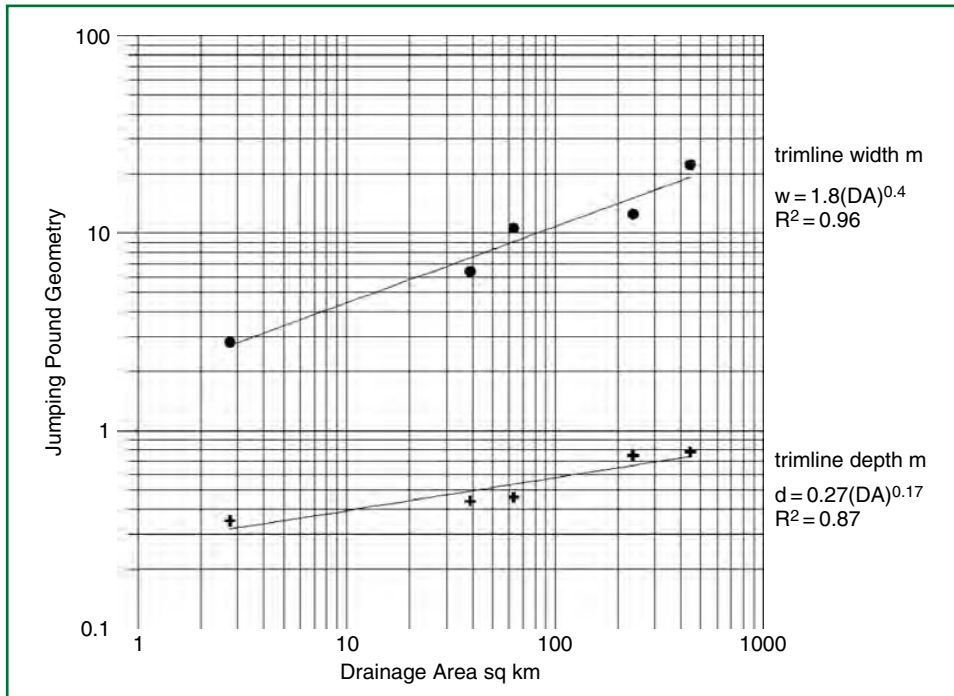
FIGURE 4.10 Reach geometry for Jumping Pound Creek AB sampled in 2003 (Biology 6183, University of New Brunswick).

A minimum of 49 samples should be taken. If present, pools and riffles should be sampled separately.

2. Determine the average bankfull cross section and slope in the reach following the Basic Mapping Method above, Steps 2 and 3.

3. Calculate the mean diameter of the substratum material measured in Step 1. Plot the mean diameter of each rock as a cumulative frequency curve (Figure 4.11).

4. Using the average slope measurement and average bankfull cross section, calculate the tractive force that would be acting on the stream bed at the bankfull stage.

5. Using Lane's tractive force versus sediment size in motion plot (Figure 4.8), determine the percent of the bed paving material that would be unstable at the bankfull stage.

D. Advanced Method 2: Streambed Stability and Shear Stress

1. At 6 to 8 sites in the reach, particularly where benthic habitats have been observed, measure the velocity of the flow at 5 to 10 increments of the depth above the stream bed. Fewer intervals may be taken at shallow sites depending on the size of the flow meter. Record the size of the substrata on the stream bed at the sample site.
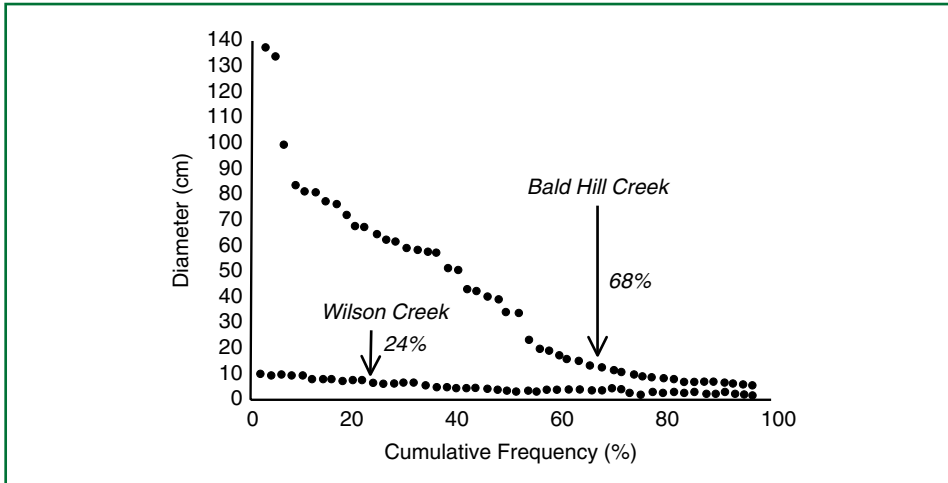
FIGURE 4.11 Cumulative frequency plot of a bed paving material sample showing the percent stable at the bankfull stage.

2. If the water is clear and shallow enough, select a range of sizes of streambed cobbles and gravels and test them on the bed at the measuring site to determine the largest size that can be transported with the present flow.
3. Plot the velocity profile at the sample sites as a function of log y (Figure 4.12).
4. Estimate the shear velocity from a line fitted to the u−log y plot.
5. Calculate the local bed shear and nominal boundary layer thickness at the measurement site.
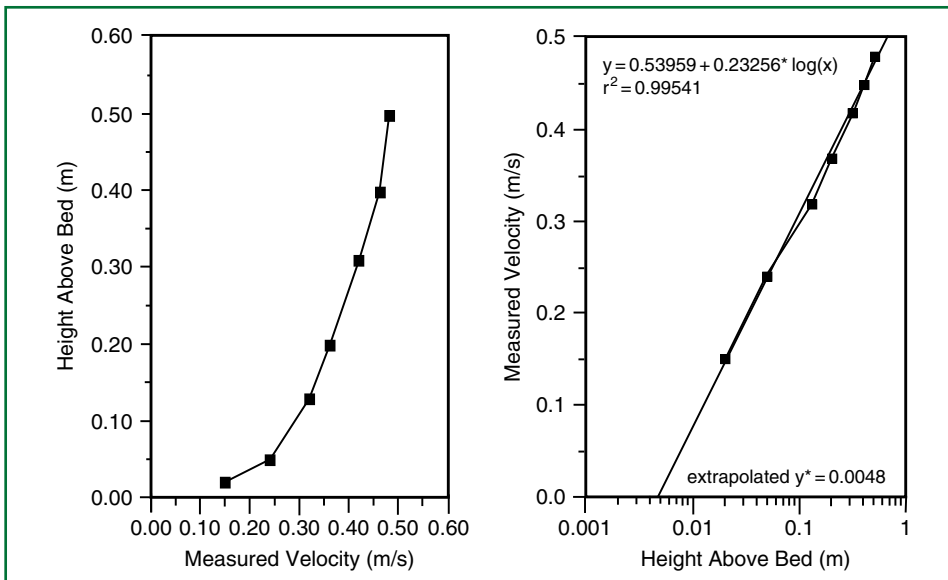
FIGURE 4.12 Sample velocity profile measurements and the velocity profile plotted as u = f(log y).

IV. QUESTIONS

1. In your mapping of hydraulic conditions and habitats, you measured the approximate area covered by each of the flow conditions mapped in the reach. How is the distribution changed between higher and lower flows?
2. In the Advanced Mapping Method you estimated the volume of flow in a critical section from the cross-sectional area and velocity, and from the upstream velocity measurements, you estimated the cross-sectional area that is contributing flow to the critical section. What advantages or disadvantages do organisms have by residing in habitats in or near the critical flow zones.
3. If there is a rapids or riffle across the reach, what portion of the total flow would you estimate passes through critical flow zones? What is the likely impact on bed stability and benthic habitats of straightening and channelizing the reach (see Newbury 1995)? How might this impact fish distribution?
4. What associations of fish and/or insects with flow and substratum conditions were observed? If available, compare the habitats to published preference curves. If fish sampling was undertaken in the study reaches (see Chapter 22), plot the species and their density versus the stream length measured on the long profile (Figure 4.13). Is there a relationship with habitat changes along the stream?
5. Using Table 4.1, calculate the Froude and Reynolds numbers of the preferred habitats of the observed species. Plot the range of observations on a velocity-depth curve. Do the ranges of preferred areas overlap? If flow data were gathered throughout the reach, estimate the percentage of available velocities and depths occupied by preferred habitats at the time of observation.
6. Discuss the anomalies found in specific reaches (geometry, position on the long profile of the stream, water temperature, groundwater influence, geological setting,
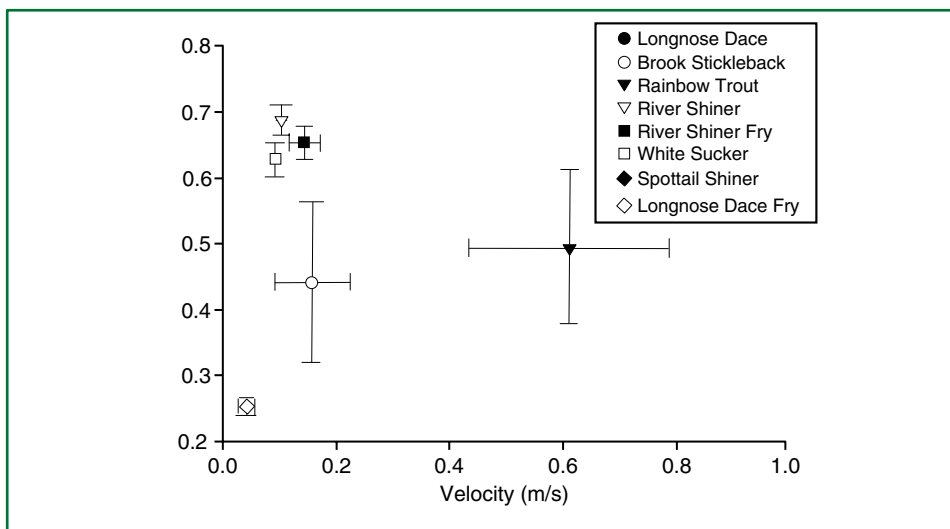

FIGURE 4.13 The range of velocities and depths observed in preferred habitats in the lower reach of the 2003 Jumping Pound Creek AB stream survey (drainage area 459 km^2) (Swanson 2003).

TABLE 4.1	Hydraulic Habitat Preferences Observed in the Lower Reach of the 2003 Jumping Pound Creek AB Stream Survey (drainage area 459 km^2) (Swanson and Ray 2003).

				Reynolds
Fish Species	Life Stage	Froude	(Depth)	(Fish Length)
Longnose Dace	Fry	0.026	10000	1000
	Adult	0.055	91420	9600
River Shiner	Fry	0.027	9200	1000
	Adult	0.038	67228	6860
Spottail Shiner	Fry	0.026	10000	1000
	Adult	0.037	54000	5400
Brook Stickleback	Juvenile	0.074	69510	6200
White Sucker	Juvenile	0.036	55000	6160
Rainbow Trout	Juvenile	0.276	303780	46970

etc.). Are there any unique habitats such as deep refugia, cool water sources, or fish passage obstacles in the anomalous reaches? Does the species composition change abruptly in or below the reach?

7. What is the largest size of bed material that could be transported at the bankfull stage? From your general mapping of the substrata, what areas of the channel bed would be in motion at the bankfull stage? Are these areas associated with any of the observed habitats?

8. What strategies must biota adopt to survive during bankfull flow events and intervening low-flow periods in the specific configuration of your sample reach?

9. Compare the local bed shear stress predicted from the average velocity or velocity profile with the general bed shear stress predicted by the tractive force. What are the differences?

10. Can insects live entirely within a laminar sub-layer thickness estimated at the velocity profile sites? See Chapter 20 and 21 and discuss the adaptations that benthic insects have to live in high shear stress areas.

11. Compare the bed shear stress predicted by the velocity measurements and by the tractive force estimate to the cobble test prediction assuming Lane's relationship applies. Discuss the consistency or reasons for discrepancies in the estimates.

V. MATERIALS AND SUPPLIES

Materials

Clipboard and drawing materials
Graph paper (arithmetic and semi-log 2 cycle)
Topographic map of the drainage basin containing the sample reaches
Triangular scale (1:50 to 1:250 scales)

Equipment

100 m flexible tape
30 m flexible tape

Calculator with power functions
Floats (e.g., oranges, wood blocks, water-filled balloons)
Meter sticks (2)
Round river cobbles ranging from 1 to 15 cm (may be on site)
Small velocity meter and rod
Stadia rod and survey level with tripod (e.g., construction site type)
Stopwatch
Waders

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STREAM SURVEY SHORT FORM: HYDROLOGY OBSERVATIONS   **SKETCH ON REVERSE**
STREAM REACH: date: party:

distance   tape
height   water level

x-sec 1

x-sec. 2

notes

Discharge Measurement: Q = sum of q's =   cu m/s

w
d
v
q

Notes:

slope: avg. bankfull width   m   avg. bankfull depth   m
present discharge: cum/s /   x-sec. area of present flow: sq m. =   avg. velocity: m/s

substrate sample
size= median diameter cm.

size
rank

median size
cm

OBSERVATIONS

1) Walk through the reach and prepare a sketch on the reverse side of this sheet showing the stream banks, riparian zone, pools, riffles, runs, and other major features in the channel.

Note the position of your x-secs, sample sites and photos on the sketch.

2) Locate 2 x-sections in the reach that typify the various flow conditions. Measure each section by stretching a horizontal tape across the channel between the bankfull or vegetation scour level (see sketch). Measure down from the tape to the streambed at several locations and write the measurements on your x-sec. diagram.

Note the distance from the tape to the water surface to establish the x-sec. of the present flow. Estimate the distance from the tape to the floodplain level on both banks if the channel is entrenched.

3) Measure the velocity of flow in several locations across the most uniform flow x-sec. in the reach. Write the width, depth and average velocity measurements in the table and calculate the present discharge.

4) Estimate the average slope of the water surface in the reach with a survey level and note it on you data sheet.

5) Measure the median axis of at least 49 bed paving materials by walking randomly through the entire reach and selecting the largest size of material at every few stops. Rank the sizes from 1 to 49.

6) Correct your sketch and check your observation sheet before leaving the reach...remove any markers.

APPENDIX 4.1   A typical summary sheet of reach survey methods and observations.

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Temperature, Light, and Oxygen

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I. INTRODUCTION

A. Temperature

Temperature is one of the most important variables in the biosphere. Temperature affects movement of molecules, fluid dynamics, saturation constants of dissolved gases in water, metabolic rates of organisms, and a vast array of other factors that directly or indirectly affect life on earth. Typically, the greatest source of heat in running waters is solar radiation, particularly where there is direct sun light over most of the water surface. Various factors can affect stream temperatures across a hierarchy of spatial scales. Small streams in forested landscapes often have a dense canopy cover that shades the stream surface resulting in cool temperatures. Opening of canopies as a result of harvesting the riparian forest can dramatically increase temperatures (Johnson and Jones 2000). Likewise, transfer of heat from groundwaters may be particularly important in governing stream temperatures (Baxter and Hauer 2000, Mellina *et al.* 2002). Regardless the direct or indirect controlling mechanisms in a particular stream, temperature plays a vital role in the presence/absence, life-histories, and spatial distribution of stream organisms (Hawkins *et al.* 1997, Lowe and Hauer 1999, Hauer *et al.* 2000, Ebersole *et al.* 2001).

Annual fluctuations in stream temperature can be very important to stream organisms Critical life history variables (e.g., reproduction, growth) of lotic plants and animals (from diatoms and aquatic insects to fish and other poikilothermic vertebrates) are regulated by temperature. Many stream animals use temperature or temperature change as an environmental cue for emergence (aquatic insects) or spawning (fishes). In temperate

regions, water temperature in winter may be at or approach 0°C, whereas summer temperatures may achieve temperatures >30°C (Hauer and Benke 1986, Lowe and Hauer 1999). Generally, streams experience *diel temperature flux*. Range in daily temperatures of more than 5°C is common (Figure 5.1). Diel temperature flux also may be very high in special environments; for example, in very small alpine streams that have direct solar radiation, afternoon temperatures in late summer may reach >20°C, whereas night temperatures approach 0°C. Even large rivers that have discharges in excess of 500 m³/s may experience diel temperature ranges of 3–5°C. However, because of the high latent heat of water (which means that adsorption or emission of a large quantity of energy is needed to change even 1°C), stream temperatures tend to vary much more narrowly on a daily basis than do air temperatures.

Temperate, arctic, and montane streams and rivers often freeze during winter greatly affecting stream discharge, light, dissolved oxygen, and many other variables. Ice may greatly disturb stream habitats and affect the distribution and behavior of stream organisms (Bradford *et al.* 2001).

Often temperature from a given stream reach is presented as a single location regime. This leads to the common misconception that stream temperatures are uniform among habitats within a stream reach. On the contrary, stream temperature may be highly variable between habitats only a few meters apart. Backwater depositional areas often are much warmer than waters in the stream channel. This would be particularly so in alluvial, gravel-bed rivers, which have high connectivity between channel waters and ground waters. Habitats receiving groundwater (see Chapters 6 and 33) may be several degrees colder in summer or warmer in winter than the main stream temperatures. Streams frequently express significant changes in temperature from small shaded headwaters to broad, open canopied river reaches. This phenomena is particularly enhanced in mountainous regions where a river may head in alpine environs, but flow through a much warmer downstream climate before confluence with other river waters, a lake, or the ocean (Hauer *et al.* 2000).

B. Light

Light is a critical variable in most ecosystems. In streams, as in all aquatic environments, solar radiation is necessary for photosynthesis by algae and macrophytes. Solar radiation is also the medium through which all visual behavior (e.g., predation by fish) is expressed. Because streams are so closely linked with the surrounding terrestrial landscape, lotic light regimes are highly influenced by terrestrial objects such as trees or geologic features. Shade created by an overhanging tree canopy restricts primary production in many streams in undisturbed forests (e.g., Hill *et al.* 1995). The longitudinal (downstream) change in light regime and its consequences for stream bioenergetics is an integral part of stream ecosystems (Vannote *et al.* 1980). Even in regions where streamside vegetation provides little shade, steep banks or canyon walls can significantly reduce the quantity of light arriving at the stream (Minshall 1978).

Although the wavelengths of solar radiation range from <300 to >5000 nm, the 400- to 700-nm range is of greatest interest to aquatic ecologists studying photosynthetic processes. This is the range of wavelengths that autotrophs use to power photosynthesis, and as a consequence, the 400- to 700-nm range is referred to as *photosynthetically active radiation* (PAR). This range of wavelengths also corresponds roughly with the range that the human eye sees (i.e., visible light). Photosynthetically active radiation is measured with quantum sensors: specially designed photocell systems that quantify the
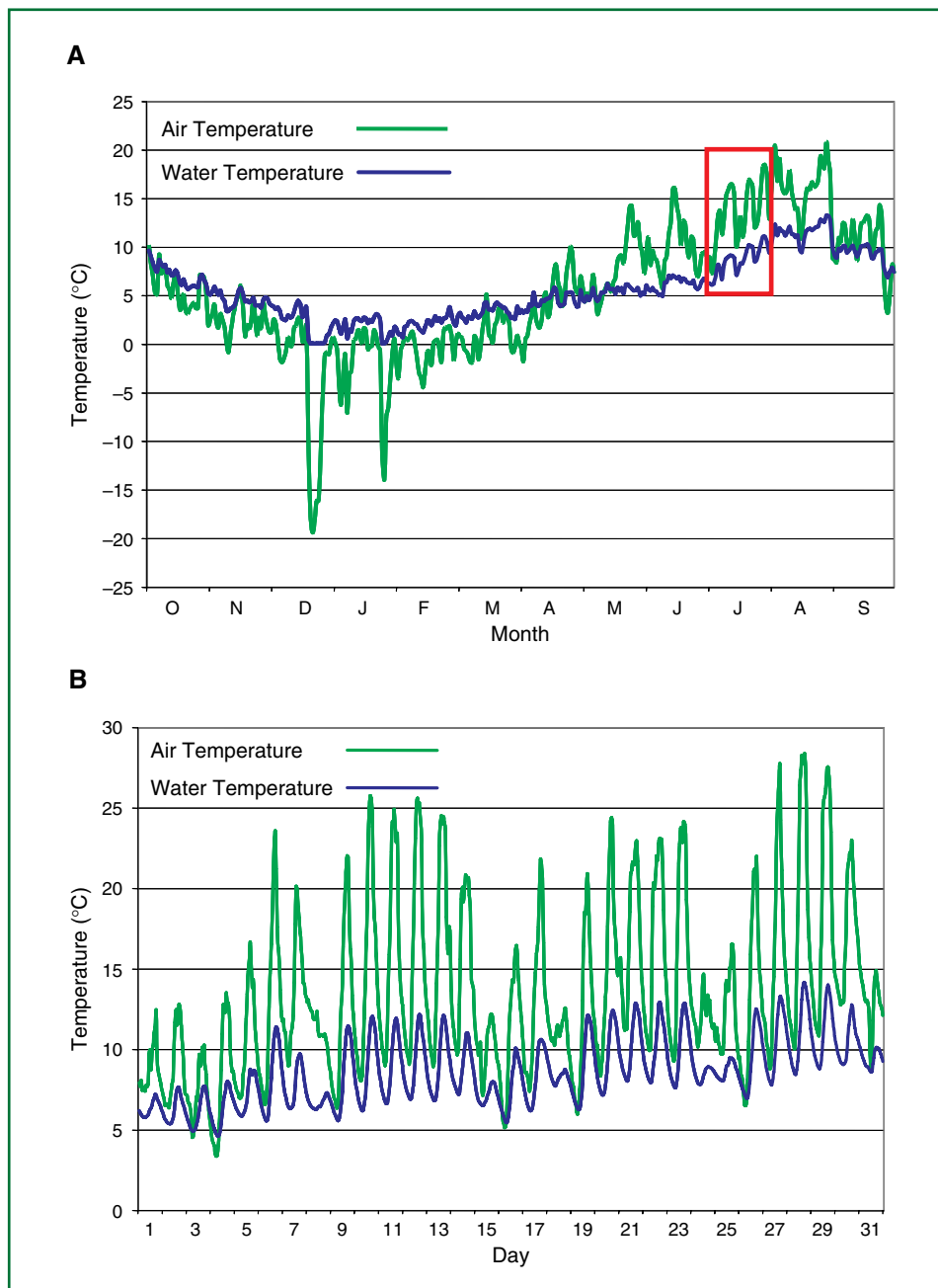
FIGURE 5.1 (A) Daily mean air and water temperatures calculated from hourly means (°C), McDonald Creek, Glacier National Park, Montana (USA), October 1, 1998, to September 30, 1999. (B) Hourly mean air and water temperatures calculated from 5 minute interval, instantaneous measures (°C), McDonald Creek, Glacier National Park, Montana (USA), July 1999 (red box in Panel A).

number of photons in the 400- to 700-nm range falling on a specific area per unit time, called *photon flux density* (PFD). Units for PFD (and PAR) are μmol quanta $m^{-2} s^{-1}$ or μEinsteins $m^{-2} s^{-1}$. The Einstein is a mole of photons, but it is not an SI unit and its use is declining. Ecological studies that integrate PAR over longer periods frequently report it as mol quanta $m^{-2} d^{-1}$.

Photon flux densities to stream ecosystems vary tremendously over time. During the course of a sunny day, PFDs in unshaded streams can range from 0 (before dawn or after dusk) to >2000 μmol quanta $m^{-2} s^{-1}$ (midday). Clouds crossing in front of the sun add variability within a day, and clouds associated with passing weather fronts create significant day-to-day variability. Seasonal variation in lotic light regime is caused by changes in sun angle and day length and by phenological changes in streamside vegetation. For example, the shading effect of vernal leaf emergence decreases PFDs at the surface of streams in deciduous forests by two orders of magnitude (Hill *et al.* 2001).

Spatial variability in lotic light regime is also high. Variation in the amount of shade cast by streamside vegetation is responsible for much of the spatial variability of light in streams. Gaps in the tree canopy above forest streams create substantial site-to-site differences in irradiance. One of the more important impacts of humans on lotic ecosystems is the alteration of light regimes caused by streamside vegetation removal. Streamside vegetation also plays an important role in the longitudinal gradient of light intensity in undisturbed streams systems. As stream size progressively increases downstream, riparian trees and bushes shade proportionally less of the stream, allowing more direct and diffuse sunlight to reach the streambed.

Once light reaches the water surface in a stream, it is attenuated by the water itself and even more importantly by substances in the water. This attenuation is minor in clear and shallow streams, so light intensity measured at the water surface in these streams is reasonably representative of the intensity at the streambed. However, some streams carry significant loads of suspended matter or are stained by dissolved organic matter (DOM). Light penetration in these streams is much reduced, particularly in deeper sections, so light measured at the surface of the water is not representative of what streambed biota experience. Underwater light meters are essential to characterizing light regimes in turbid or highly colored streams. Light attenuation measured in these streams can be described by the same exponential function used by limnologists and oceanographers: $E(z) = E(0)e^{-kz}$, where $E(z)$ and $E(0)$ are the irradiances at z depth and at the surface, respectively, and k is the attenuation coefficient (Kirk 1994).

An increasing amount of attention has been focused on the role of ultraviolet radiation (wavelengths <400 nm) in aquatic ecosystems during the last 15 years. Both ultraviolet A (320–400 nm) and ultraviolet B (280–320 nm) wavelengths have been attributed with deleterious effects on stream algae and invertebrates (e.g., Bothwell *et al.* 1994, Kelly *et al.* 2003) in clear, unshaded, and shallow streams. Ultraviolet light is strongly absorbed by DOM, and there is interest the both protective function of DOM and the effect of ultraviolet light in making DOM more available to bacterial metabolism (e.g., Brisco and Ziegler 2004).

C. Oxygen

Dissolved oxygen (DO) directly affects aquatic life through O2 availability and metabolism, but also indirectly through various biogeochemical processes. In most unpolluted streams and rivers, DO concentrations remain well above 80% saturation. Solubility

of oxygen increases nonlinearly as temperature decreases and decreases with decreasing atmospheric pressure associated with different altitudes or barometric change of weather.

Dissolved oxygen concentrations are not uniform within or between stream reaches. Up-welling of interstitial waters of the hyporheic zone (see Chapters 6 and 33) or side flow of ground waters may create patches of stream bottom where DO is significantly less than that of surrounding waters. Nearly all stream organisms are sensitive to oxygen concentration. Organic pollution, such as that associated with municipal sewage treatment discharge or industrial wastes, may significantly reduce DO concentrations in entire stream reaches as microbial processes consume the oxygen from the water; this is generally referred to as *biochemical oxygen demand* (BOD). In unpolluted running waters, oxygen concentration may also change dramatically between habitats. Microbial activity within leaf packs and debris dams may reduce oxygen concentrations at the microhabitat level. Streams and rivers that support luxuriant algal growth may experience broad daily ranges in DO as photosynthesis increases oxygen concentration during the day and respiration reduces oxygen concentration at night. Whole-ecosystem metabolism in streams using measures of oxygen change have been used to estimate gross primary productivity, respiration and net primary productivity (Mulholland *et al.* 2001). These variables, along with the ecological significance of P:R ratios, are also discussed in Chapter 28.

II. GENERAL DESIGN

A. Temperature

While temperature is most simply measured with standard mercury thermometers, because of the risk of breakage in the field and the resulting contamination of a site with elemental mercury, we strongly advise that researchers avoid their use in the environment. Inexpensive, alcohol-based thermometers are readily available from most scientific supply companies. Some suppliers offer field thermometers with metal or plastic jackets that protect the glass rod and thus reduce breakage. Unfortunately, inexpensive thermometers lack both precision and accuracy; generally measurement is only to within 0.5°C and often several degrees in error. For precise thermographic work, temperature measuring and recording devices should have at least a resolution of 0.1°C.

Contemporary approaches to the measurement of temperature in streams most commonly use electronic thermistors or thermocouples. A thermistor is a semiconductor that changes resistance with temperature. It is now the most commonly used electronic temperature measuring device. Most thermistors are quite small, typically consisting of a 2- to 3-mm round blob of solder on the end of two wires. The probe portion of the instrument is submerged at the location that the temperature reading is desired. The probe generally consists of a plastic covered metal that decreases in electrical resistance with increasing temperature. The probe is connected to a battery-operated analog or digital recorder that displays the temperature. Thermistor-type thermometers are generally very accurate ($\pm$0.1°C).

The thermocouple-type temperature probe uses the phenomenon that a voltage is spontaneously generated between the ends of an electrical conductor that passes through a temperature gradient. If two different conducting wires pass through the same temperature gradient, then the voltage developed will be different in each wire. Because the thermocouple only detects a temperature difference, a thermistor (usually) at the voltage measuring instrument is required to define the baseline from which the temperature at the remote end can be calculated. The technical advantage of a thermocouple is that they

are voltage generators with a very low internal resistance, which makes them resistant to electrical interference even over long cable runs.

Data acquisition systems designed for permanent field deployment to record data from various environmental probes have been available for several decades (e.g., Campbell Scientific Inc.). Probably the most common variable measured by these recorders is temperature. However, with the relatively recent advent of micro-computerization and nano-sensors, "stand-alone" temperature recorders containing a thermistor, battery, computing chip and a means of communicating to a personal computer have proliferated over the past decade. These field instruments use a thermistor-type thermometer and record temperature at user-defined and programmed time intervals. In all cases, these single or dual variable (also some contain a pressure transducer to measure water depth) data loggers permit downloading of the data into easily handled computer data files. These devices are available in a variety of sizes and different levels of rugged design. For stream research, we recommend obtaining units that are specifically designed to hold-up under the rigors of running water systems. Although we are not endorsing any particular product, we have successfully and routinely used the Onset Hobo Water Temp Pro and StowAway Tidbit Miniature data loggers, and the Vemco Minilog 12 data logger. These units are in the $100 to $150 range.

Any temperature measuring device, whether a simple handheld thermometer or a thermistor or thermocouple probe or data logger, should always be calibrated regularly against a precision thermometer certified by the National Institute of Standards and Technology (NIST, formerly the National Bureau of Standards). This should be done in the laboratory before deployment of either hand-held or extended-time temp-loggers.

Thermal infrared cameras were originally developed for various types of military applications. More recently, forward-looking infrared (FLIR) cameras have been used for nonmilitary and research purposes. Light, simple, and relatively inexpensive thermal imaging cameras can be deployed from aircraft (Figure 5.2) to obtain thermal emission data over entire river reaches or may be deployed at a single location and used over an extended time to obtain temperature variation along a specific channel segment. While thermal imaging allows broad area analysis, it has the disadvantage of being restricted to measuring emission radiation and thus can only measure surface temperatures.

B. Light

Light sensing devices range in sophistication from semiquantitative ozalid paper meters to spectroradiometers that measure light intensity at individual wavelengths and cost over $15,000. For many ecological investigations, quantum sensors are the appropriate measuring device, especially if the study focuses on photosynthetic organisms or processes. Underwater quantum sensors rugged enough to be deployed on the stream bed can be constructed from readily obtained materials for as little as $15 (Melbourne and Daniel 2003). Commercially available quantum sensors suitable for above-water measurements of PAR cost $150–$350, and commercially available quantum sensors suitable for measuring underwater PAR cost as much as $840 (including the cost of an underwater cable). Other photocell sensors that measure irradiance instantaneously, but at different wavelengths, include pyranometers and photometers. Pyranometers measure a broader range of wavelengths (400–1100 nm) than quantum sensors; pyranometer measurements are reported in energy units per area, usually Watts/m^2. Photometers measure approximately the same range of wavelengths as quantum sensors, but their sensitivity is weighted to match that of the human eye, which is most responsive to green-yellow
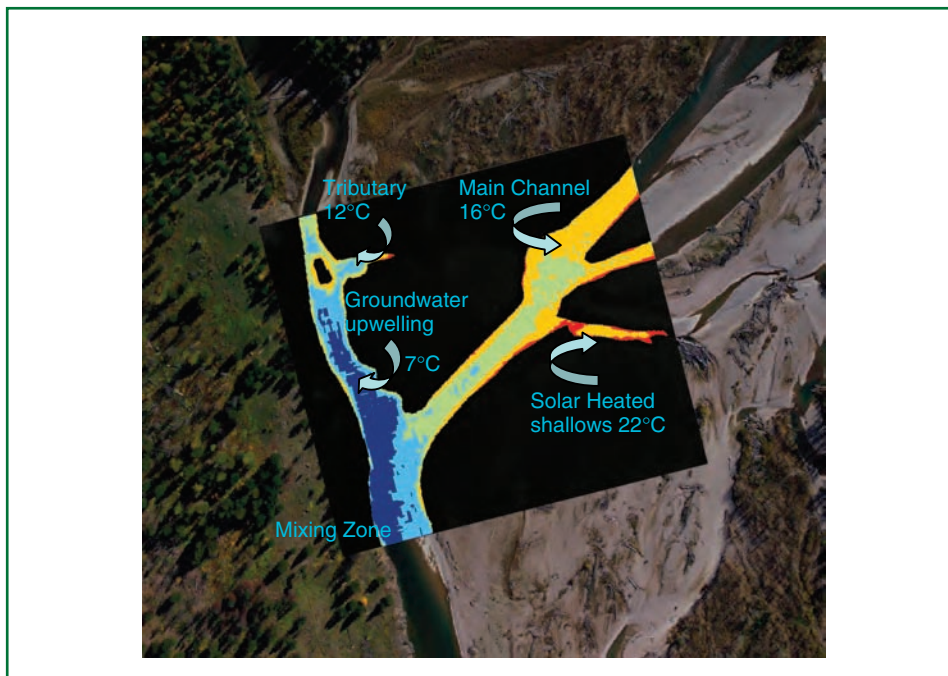
FIGURE 5.2 Thermal infrared (near IR) image classified by temperature and superimposed on an aerial image of the Nyack floodplain, Middle Fork Flathead River in Montana. Image illustrates the spatial variation in temperature distributed across a river landscape and the utility of IR imagery in landscape scale study.

wavelengths (ca. 500–600 nm). Photometer measurements are reported in footcandles or lux (SI units). Pyranometers and photometers are generally less expensive than quantum sensors. Although quantum sensors are the preferred light-sensing device for most studies, light measurements made by quantum sensors, pyranometers, and photometers are usually quite highly correlated.

Instantaneous measurements of light intensity made once or only a few times during the day are better than no measurements at all, but they fail to represent the daily dynamics of light. Light measurements made at frequent intervals are much more representative of the light regime experienced by stream biota than "spot" measurements. Most light sensors can be connected to dataloggers to record light intensities at intervals chosen by the investigator. If these intervals are frequent enough (e.g., ≤15 minutes), a reasonable estimate of primary production can be made by using the data in photosynthesis-irradiance models (e.g., Hill *et al.* 2001). Integrating the interval-specific light intensities over the course of a day provides a daily integrated light intensity (e.g., moles of quanta per m^2 in the case of quantum sensors) that is commonly used to quantify light regimes. In geographical locations marked by day-to-day variations in cloud cover, daily integrated light intensities are useful data.

Pyrheliometers and ozalid paper meters can be used when an instantaneous measurement of irradiance is not needed. Pyrheliometers measure the light energy absorbed by a black metallic surface, and their measurements, similar to those recorded by the pyranometer, are expressed in energy units (W/m^2). They have been used extensively in limnology and oceanography, but measure a very broad range of wavelengths (300–5000 nm)

that includes ultraviolet and infrared wavelengths, and have a slower response time than sensors relying on photocells (e.g., quantum sensors, pyranometers, photometers), Pyrheliometers are best suited for monitoring solar energy at a single location. Ozalid paper meters provide an estimate of time-integrated photon flux. They are constructed by the investigator from plastic petri dishes and light-sensitive blueprint (ozalid) paper, which strongly absorbs wavelengths around 410 nm (Friend 1961). Although the ozalid paper meter is sensitive to only a small portion of the PAR range, it can be calibrated against quantum sensors, providing a modestly accurate estimate of PAR as long as the radiation spectrum used to calibrate the sensors is roughly similar to that where the sensors are used. However, the overall usefulness of ozalid paper meters is constrained by the relatively low sensitivity and poor precision. The best choice for obtaining integrated light measurements is a photocell-type sensor attached to a data logger programmed to record readings at intervals appropriate for the particular study questions. Advances in electronics have resulted in smaller, cheaper data loggers; one underwater unit sold by Onset for <$50 incorporates a photometer and a temperature sensor with a 64K data logger.

Instruments for measuring ultraviolet radiation include broadband meters and spectroradiometers. Broadband meters are relatively cheap, but as their name suggests, integrate over a relatively large range of the UV spectrum (280–400 nm). This breadth of range is problematic because UV effects are highly dependent on wavelength (smaller wavelengths are much more damaging than higher ones). Spectroradiometers measure the intensity of individual wavelengths (including those in the visible range), and are therefore preferred. Unfortunately, spectroradiometers are quite expensive. Commercially built underwater spectroradiometers cost $15,000 or more. One less expensive option (~$5000) is custom-built (Ocean Optics) irradiance probes connected by fiber-optics to a streamside spectrometer run by a laptop computer (Frost *et al.* 2005) to reduce cost. Another possible avenue to measure UV radiation involves the use of polysulfone plastic, which absorbs radiation in the 290–320 nm ultraviolet range and has been used to estimate UVB in wetlands (Peterson *et al.* 2002).

C. Oxygen

Dissolved oxygen is generally measured using either of two methods; the Winkler Method or the Membrane-Electrode Method (APHA *et al.* 1998). Each method has specific advantages and disadvantages. The advantages of the Winkler Method are (1) when performed by experienced persons it can very accurately measure DO with great precision, and (2) it is relatively inexpensive to acquire the necessary titration burettes, sample bottles, and chemicals. The primary disadvantages of the Winker Method are (1) one cannot continuously monitor change in DO, but rather must rely on discrete measures, and (2) reducing or oxidizing materials dissolved in the water can interfere with accurate measurement of DO concentration. The advantages of the Membrane-Electrode Method are (1) ease of use, and (2) one can continuously monitor change in DO, especially in running waters that move water across the probe membrane. The primary disadvantage of the Membrane-Electrode Method relates to difficulties associated with instrument maintenance and calibration. The cost of oxygen measuring instruments has gone down significantly in the past decade. Where once a recording device and probe cost over $1500, a YSI Model 550A Dissolved Oxygen Meter (Range: 0 to 20 mg/L (0–200%); (±2% air sat.) or ±0.3 mg/L (±2% air sat.), is now available for under $500.

III. SPECIFIC METHODS

Basic Method 1: Winkler Method Determination of Dissolved Oxygen Concentration

Laboratory Preparation of Reagents

Manganous sulfate solution

1. Dissolve 364 g $MnSO_4 \cdot H_2O$ in distilled water, filter through a 1.0 μm glass-fiber filter, and dilute to 1 L.

Alkali-iodide-azide reagent

1. Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water and dilute to 1 L.
2. Dissolve 10 g NaN_3 in 40 mL distilled water and add to NaOH and NaI solution.

Sulfuric acid

1. Fill a small glass bottle with conc. H_2SO_4.

Starch

1. Dissolve 2 g laboratory grade soluble starch in 100 mL hot distilled water.
2. Add 0.2 g salicylic acid as a preservative if the starch solution will be kept for more than 48 hr.

*Standard sodium thiosulfate titrant*

   $(0.025 M \ Na_2S_2O_3)$

1. Dissolve 6.205 g $Na_2S_2O_3 \cdot 5H_2O$ in ≈900 mL distilled water.
2. Add 1.5 mL 6*N* NaOH or 0.4 g solid NaOH and dilute to 1000 mL.

Collection of Sample

1. Collect samples very carefully in narrow-mouth, glass-stoppered, 300 mL BOD bottles.
2. Avoid entraining or dissolving atmospheric gases during sampling. This can be accomplished by using a large-mouth beaker or if a particular microhabitat is to be sampled, one may use a large (>100 mL) plastic syringe to draw water from a specific location in the stream.
3. Fill the BOD bottle to overflowing by 2–3 X the volume of the bottle. Prevent turbulence and bubbles during filling of either the sampling device or the BOD bottle.
4. Stopper the BOD bottle by carefully tipping the bottle slightly and inserting the glass stopper making certain no gas bubbles are entrained in the bottle and immediately proceed to the analysis.

Analysis Procedure

1. Add 1 mL $MnSO_4$ solution to 300 mL BOD bottle filled with sample water using a glass pipet.
2. Immediately follow by adding 1 mL of the alkali-iodide-azide reagent using separate pipet.
3. Stopper carefully to exclude air bubbles.
4. Mix the sample and reagents by inverting the sample bottle several times.
5. A brown precipitate $[MnO(OH)_2]$ will form in the presence of dissolved oxygen in the sample water.

$$MnSO_4 + 2NaOH \rightarrow Mn(OH)_2 + Na_2SO_4$$
$$2Mn(OH)_2 + O_2 \rightarrow 2MnO(OH)_2$$
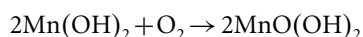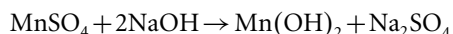
6. When the precipitate has settled to the bottom 1/3 of the bottle, add 1 mL concentrated sulfuric acid with a glass pipet.
7. Restopper the BOD bottle and mix by inverting several times until the precipitate is completely dissolved resulting in the liberation of iodine in direct proportion to the concentration of dissolved oxygen.

$$MnO(OH)_2 + 2H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O$$
$$Mn(SO_4)_2 + 2NaI \rightarrow Mn(SO_4) + Na_2SO_4 + I_2$$
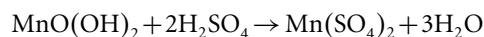
8. The quantity of iodine present is determined by titrating 200 mL of the sample with $0.025\,M$ $Na_2S_2O_3$. Titrate a volume corresponding to 200 mL of the original sample by correcting for losses as a result of addition of reagents. Thus, for a total of 1 mL $MnSO_4$ and 1 mL alkali-iodide-azide reagents added to a 300 mL sample, titrate:

$$200 \times 300/(300-2) = 201.3\,mL$$

9. Titrate to a pale straw color. Add a few drops of starch solution forming a blue color. Continue to titrate carefully and slowly to the first disappearance of blue color. Record the volume of titrant used. Note that after a minute or so a pale blue color may return; however, this should not be titrated.

Calculation

1. For titration of 200 mL of sample (201.3 mL of end product), 1 mL of $0.025\,M$ $Na_2S_2O_3 = 1$ mg/L Dissolved Oxygen.

Basic Method 2: Spatial Variation of Temperature, Light, and Dissolved Oxygen

1. Choose sections of a study stream that have readily apparent differences in landscape, channel form, and/or groundwater interactions (see Chapters 1, 2, 6, and 33). Select specific locations that also capture the range of variation in light from being relatively open to heavily shaded by streamside vegetation or by geophysical features. Study stream sections should consist of at least one riffle-pool-run sequence, if possible. Within each stream section select and mark at least five cross-stream transects. The distance between transects will depend upon the length of each section, but transects should be at least several meters apart and intersect different and representative habitat types.

2. Measure temperature, light, and oxygen at a series (9–15) of equidistant points across each transect, including points at both edges of the stream. Measure water temperature at the surface and as close to the stream substratum as possible. While measuring light, if using a quantum sensor or other instantaneous sensor (i.e., pyranometer, photometer), measure and record the photon flux just above the water surface at each point on the transect. For this approach, oxygen is most easily measured with an oxygen probe. As with temperature, DO should be measured near the surface and at the stream bottom.

3. Begin measurements at the downstream transect and work across each transect before moving to the next upstream transect. Measure and record as quickly as possible to reduce the confounding effects of temporal variation. Make note of changing cloud cover during the measurements.[1] If an underwater sensor is available, measure light at 10 or 20 cm depth intervals at a single deep site to obtain an estimate of light attenuation with depth.

4. For each transect graph temperature, light, and DO versus transect position (m) for each data point. Conduct an analysis of variance (ANOVA) to determine whether there is greater variation between points within transects or between transects for each section for each of the three variables.

5. Stratify each data collection point across each transect into habitats (e.g., riffle, pool, thalweg, bank margin). Combine temperature data from each habitat type. Calculate mean, standard error, and coefficient of variation for each habitat type; compare habitats. Do the same for DO data.

6. Combine light data from each section into paired frequency histograms labeled open and shaded; use a doubling scale for the *x*-axis, (e.g., 0–10, 11–20, 21–40, 41–80, 81–160, 161–320, and >321 μmol quanta m^{-2} s^{-1}) (see example, Figure 5.3). Calculate mean, standard error, and coefficient of variation for each section; compare open canopy and shaded stream sections. If underwater light

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[1] If ozalid-paper light meters are used instead of instantaneous meters, then glue (gel superglue or silicon sealant) individually labeled ozalid meters on the tops of steel rods (e.g., 3/8–1/2 inch rebar) driven into the stream bottom at each point on each transect. Uncover the aperture of the meters in timed sequence; the same timed sequence should be followed when the meters are collected later. Allow at least 1–2 hours for Ozalid paper exposure, and be sure to retain the identity (section, transect, point) of each meter when developing and reading the meters in the laboratory.
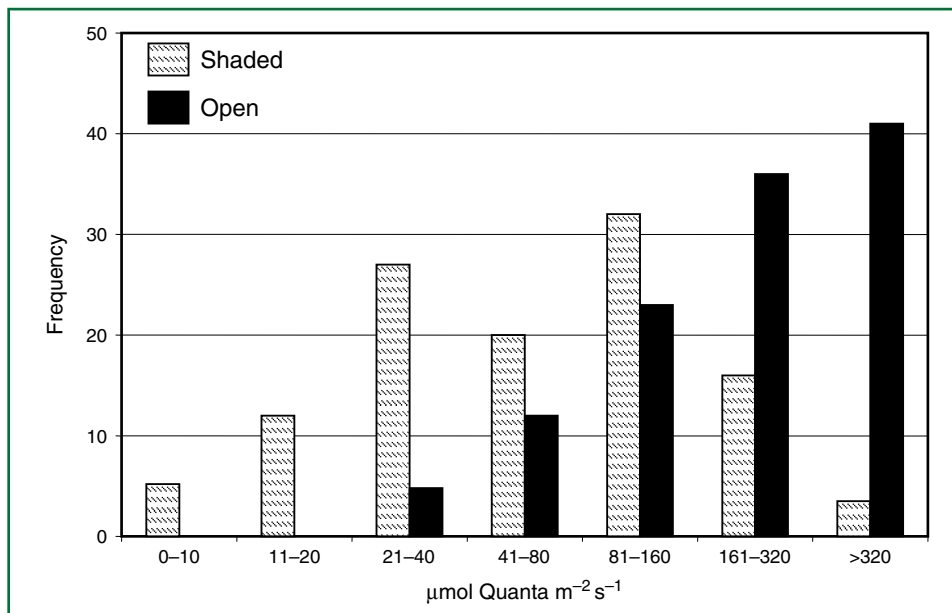
FIGURE 5.3 Example of paired frequency histograms of light intensity (μmol quanta m^{-2} s^{-1}) in relatively shaded and open canopy stream reaches.

measurements were made, calculate the vertical attenuation coefficient k in the exponential equation:

$$E(z) = E(0)e^{-kz} \tag{5.1}$$

where $E(z)$ and $E(0)$ are the irradiances at a depth of z meters and just below the surface, respectively (Kirk 1994).

Advanced Method: Detailed Temporal and Spatial Variation of Temperature, Light, and Dissolved Oxygen

1. Locate a series of representative sample sites within the study reach. Sites should be chosen to represent the range of variation of habitats within the reach.
2. Capture of long-term data sets will generally require the deployment of electronic data logging equipment. Most data loggers can be equipped with temperature probes, quantum sensors, and DO meters.
3. Depending on the size of the stream or the habitat that is being studied, temperature, light and DO can change dramatically and rapidly. Hourly data is likely a minimum of frequency for the collection and recording of these data. Several data loggers have the capability of collecting data at more frequent intervals

(e.g., 5 min) and then averaging these data and recording the average each hour. This approach has many advantages over taking single hourly readings.
4. Plot stream temperature, irradiance and DO versus time of day. Plot temperature versus light, oxygen versus temperature, and oxygen versus light.

IV. QUESTIONS

1. Identify the spatial variation in temperature, light, and DO in the study stream. What appears to be the sources of variation? Are these sources different between these three variables? Are the sources random or predictable?
2. Many investigations characterize stream temperature, light regime, and concentration of DO at a particular site in a stream based on measurements taken at a single point. Based on your data, how accurately would a measurement at a single point reflect these conditions for the stream section(s) you chose? If you measure light at only one time, when should it be done?
3. Assume that photosynthesis by stream algae is limited by insufficient light below $200\,\mu$mol quanta $m^{-2}\,s^{-1}$. At what percentage of sites or times is photosynthesis light-limited? What if photosaturation irradiance was reduced to $100\,\mu$mol quanta $m^{-2}\,s^{-1}$?
4. Is there a relationship between temperature and oxygen? What about light and oxygen? Is the relationship linear? Why or why not?
5. What are the depths at which light is attenuated to 50%, 10%, and 1% of surface intensity? How might this change throughout the year? Do you think that light attenuation with depth affects algal photosynthesis at your study site? Why or why not?

V. MATERIALS AND SUPPLIES

Field equipment for stream transects

> Field notebook
> Measuring tapes
> Transect markers (rebar, plastic flagging, etc.)

Temperature

> Electronic thermistor ($\pm0.1°C$)

Light

> Quantum sensor (preferred), pyranometer, or ozalid paper

Oxygen

> *Winkler Method*

> 300 mL BOD bottle(s)
> 500 mL beaker
> Burrett stand
> Pipettes to dispense reagents
> Reagents

Starch bottle with eye dropper
Titration burrett

Probe Method

Oxygen meter with probe

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Hyporheic Zones

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I. INTRODUCTION

The *hyporheic zone* is a portion of the groundwater interface in streams where a mixture of surface water and groundwater can be found. Original use of the term can be found in the work of Orghidan (1959), who described the interface as a new groundwater environment containing a distinctive biota. The word hyporheic derives from the Greek words for flow or current (*rheo*) and under (*hypo*). Hyporheic zone waters can be found both beneath the active channel and within the riparian zone of most streams and rivers. Interest in this dynamic interface or ecotone has grown substantially (e.g., Stanford and Simons 1992, Valett *et al.* 1993, Findlay 1995, Jones and Holmes 1996, Brunke and Gonser 1997, Morrice *et al.* 1997, Boulton *et al.* 1998, Woessner 2000, Edwards 2001, Malard *et al.* 2002, Hancock *et al.* 2005), after Danielopol (1980) and Hynes (1983) argued forcefully for better integration of groundwater and stream research.

When this chapter was first written and published (Dahm and Valett 1996), there were 60 papers in the peer-reviewed literature where the term *hyporheic zone* appeared in the abstract or keywords. The number of peer-reviewed papers from 1995 to the middle of 2005 with *hyporheic zone* in the abstract or keywords totals 365. In fact, there were 47 papers on this topic in 2002, 55 papers in 2003, and 54 papers in 2004. Fields such as ecology, hydrology, geomorphology, geochemistry, fisheries, environmental engineering,

and contaminant transport have embraced the concept of the hyporheic zone as a dynamic and distinguishable interface between surface waters and groundwaters.

In a broad sense, the hyporheic zone can be defined as the saturated sediments within and associated with streams and rivers in which surface water and groundwater mix. Triska *et al.* (1989) provide an empirical perspective of this interstitial environment by recognizing a surface hyporheic zone where >98% of the water was recently advected from the channel and an interactive hyporheic zone where there is >10% but <98% channel water. Vervier *et al.* (1992) prefer a definition that emphasizes the ecotonal nature of the hyporheic zone, in which they stress that the hyporheic zone is an ecotone between surface water and groundwater, where boundaries are spatially and temporally dynamic. Brunke and Gonser (1997) also view the hyporheic zone as an ecotone between river and groundwater ecosystems that can be characterized by hydrologic, chemical, zoologic, and metabolic features. Boulton *et al.* (1998) also define the hyporheic zone as an active ecotone between surface stream water and groundwater, where water, nutrients, and organic matter are exchanged. Inherent in these definitions are the concepts of an ecotone or continuum that is heterogeneous and varies both temporally and spatially. With these perspectives, important attributes of the hyporheic zone are (1) the integration of groundwater (flow through porous medium) and channel water (free flow) and (2) the associated gradients in such variables as temperature, redox potential (E_h), pH, organic matter content, microbial numbers and activity, and availability of nutrients and light. In general, key components of the hyporheic zone derived from the various definitions are the spatial and temporal exchange of channel water with the associated riverine/floodplain sediments.

It is important for stream ecologists to consider the hyporheic zone when studying streams and rivers. One reason is that this zone is an important habitat for numerous aquatic organisms. Hyporheic zones contain a wide variety of subterranean fauna and zoobenthos, either at various stages of their lives or throughout their life histories (e.g., Coleman and Hynes 1970, Stanford and Gaufin 1974, Williams 1984, Stanford and Ward 1988, Williams 1989, Boulton *et al.* 1992, Smock *et al.* 1992, Stanley and Boulton 1993, Boulton *et al.* 1997, Dole-Olivier *et al.* 1997, Brunke and Gonser 1999, Malard and Hervant 1999, Brunke *et al.* 2003, Malard *et al.* 2003a, Malard *et al.* 2003b, Olson and Townsend 2003, Olson and Townsend 2005). Much of this fauna is inadequately described and identified, and new organisms and adaptations to subterranean life are frequently being found. In addition, early research on the hyporheic zone focused on fish reproduction as fish eggs are commonly incubated in this environment (e.g., Pollard 1955, Hansen 1975, Johnson 1980, Baxter and Hauer 2000). Recent advances in understanding the role of the hyporheic zone add significantly to our fundamental understanding of stream ecology and greatly expand the documented physical space that aquatic organisms inhabit, and the region where biotic interactions and production occur. For many streams and rivers, subterranean invertebrate production in the hyporheic zone rivals or exceeds that of the benthos (e.g., Stanford and Ward 1988, Smock *et al.* 1992). Although difficult to access (Palmer 1993), hyporheic zones hold fascinating biota contained within a truncated functional biodiversity due to the lack of primary producers and limited numbers of top predators (Gibert and Deharveng 2002; Chapters 14, 19, 20 and 33). In addition, these biota also hold significant insights to stream and river ecology and overall water quality. For example, land use effects and human activity strongly influence the biota of hyporheic zones (e.g., Brunke and Gonser 1997, Boulton *et al.* 1997).

A second reason for including the hyporheic zone in studies of stream and river ecosystems is the impact that hydrologic exchange with this zone has on surface stream

biota (Chapter 33; Boulton 1993). Hyporheic zone sediments and waters are metabolically active with complex patterns of nutrient cycling, which vary spatially and temporally (e.g., Grimm and Fisher 1984, McDowell *et al.* 1992, McClain *et al.* 1994). Upwelling waters from the hyporheic zone can deliver limiting nutrients to the stream channel that influence rates of algal primary production, the composition of benthic algal assemblages, and the recovery of stream reaches after disturbance (Valett *et al.* 1990, 1994, Coleman and Dahm 1990, Pepin and Hauer 2002). Stanford and Ward (1993) have described how discrete localized zones of upwelling of hyporheic waters can produce patches of increased biotic productivity within oligotrophic riverine-floodplain ecosystems. Hendricks and White (1988) and Fortner and White (1988) have pointed out how advective water movement in the hyporheic zone affects the distribution of aquatic macrophytes in some streams. Interchange of waters between groundwater and surface water can play a major role in the structure and function of the benthic interface in streams and rivers.

A third reason for studying the hyporheic zone is the importance of this ecotone in the uptake of solutes and on ecosystem metabolism (Chapters 8 and 33). For example, rates of both nitrogen and phosphorus cycling are strongly influenced in many streams by processes occurring in the hyporheic zone (e.g., Chapter 33; Valett *et al.* 1996, Valett *et al.* 1997, Mulholland *et al.* 1997, Cirmo and McDonnell 1997, Hedin *et al.* 1998, Dahm *et al.* 1998, Dent *et al.* 2001, Hall *et al.* 2002, Thomas *et al.* 2003). Stream metabolism also is strongly affected by hydrologic exchange between surface waters and groundwaters (e.g., Jones *et al.* 1995, Jones 1995, Pusch 1996, Fischer *et al.* 1996, Fuss and Smock 1996, Naegeli and Uehlinger 1997, Fellows *et al.* 2001, Crenshaw *et al.* 2002) and the residence time of water in the hyporheic zone (Hoehn and von Gunten 1989, Brunke and Gonser 1999). Metabolism rates in hyporheic zones are closely linked to DOC dynamics (Fiebig 1995, Battin 1999, Baker *et al.* 1999, Baker *et al.* 2000, Sobczak and Findlay 2002, Clinton *et al.* 2002) and the availability of particulate organic matter (Battin *et al.* 2003) at this ecotone. End-member mixing analysis based on conductivity indicates that about 40% of hyporheic zone respiration comes from DOC with the rest supported by entrained particulate organic carbon for White Clay Creek, Pennsylvania (Battin *et al.* 2003). Metabolism that depletes dissolved oxygen concentrations also impacts hyporheic zone organisms as reviewed by Malard and Hervant (1999). Finally, the cycling of nutrients and organic matter in hyporheic zones also affects riparian vegetation along stream corridors. Harner and Stanford (2003) show faster cottonwood growth in nutrient-rich upwelling zones, and Schade *et al.* (2005) track the movement of hyporheic zone nutrients into riparian tree species using stable isotopes. Hyporheic zones can play major roles in nutrient cycling, carbon metabolism, and riparian plant growth in streams.

A fourth reason for studying the hyporheic zone is in developing, refining, and validating hydrologic models that represent the dynamics of water and solute exchange at the interface between streams and groundwaters. Modeling studies of the exchange between channel water and the hyporheic zone often employ the numerical One-dimensional Transport with Inflow and Storage (OTIS) transient storage model developed, upgraded, and maintained by the U.S. Geological Survey (see Chapter 8 and http://co.water.usgs.gov/otis/). The model employs a transient storage component to estimate exchange between channel water and the associated hyporheic zone. This modeling approach is widely used and is described in detail in Chapter 8. There are limitations, however, to this model and the stream tracer approach. Harvey *et al.* (1996) show that the stream tracer approach does not reliably characterize hyporheic exchange at higher flows, and they argue that short-term (scale of hours) exchange flows were best characterized by the transient storage model, while longer-term flow paths were not well

characterized. Runkel *et al.* (1998) describe a modeling framework that allows analysis of transient storage in streams with unsteady flows. Choi *et al.* (2000) compare a two-storage zone model to the one-storage zone model used by OTIS and conclude that the single-storage compartment model adequately characterizes dominant processes of solute retention in most cases. Haggerty *et al.* (2002) and Gooseff *et al.* (2003a and b) show that hyporheic residence time distributions fit a power-law with a very long tail (long residence times). This implies that hyporheic zones have a very large range of exchange timescales (hours to months). Runkel (2002) recommends the use of a new metric for determining the importance of transient storage in streams. This metric examines the fraction of the median travel time for stream water that is due to transient storage in a stream reach. Hydrologic retention in hyporheic zones within a reach of stream also can change over the scale of years with changing stream geomorphology and hydrology. Harvey *et al.* (2003) show that geomorphic and vegetative changes over a five-year period of decreasing flow increased the size of the hyporheic zone and average residence time of water in the hyporheic zone. The dynamics of surface water and solute interactions with the hyporheic zone result in a continuum of exchange processes and residence times that is inherently heterogeneous both spatially and temporally.

Finally, landscape characteristics and scaling of exchange processes between streams and hyporheic zones are important to material storage and transport, stream biota, and ecosystem processes. Stanford and Ward (1993) proposed the concept of a hyporheic corridor with groundwater communities and processes that vary predictably from headwater to the sea as a function of the occurrence of unconfined floodplains. The spatial distribution of these unconfined floodplains and hyporheic zones control bioproduction within channels of many streams and rivers and groundwater food webs add to riverine species richness and mediate mass transfer of bioavailable materials through floodplain ecosystems. Brunke and Gonser (1999) show that the ratio of particulate organic carbon to total fine particles explains 61% of the variation of hyporheic invertebrates in a gravel-bed stream in Switzerland. Brunke and Gonser (1997) describe local controls on upwelling and downwelling waters by geomorphic features with larger scale exchange processes linked to geological properties of the catchment. Baxter and Hauer (2000) also describe how geomorphology constrains hyporheic exchange at scales of valley segments, reaches, and channel units. Wroblicky *et al.* (1998) show that the size of the hyporheic zone is sensitive to discharge, alluvial sediment size, and bedrock lithology. Woessner (2000) describes gaining, losing, flow-through, and parallel-flow reaches of streams. Hydraulic head distribution, groundwater flow directions, stream hydraulics, channel bed form, and hydrogeologic parameters are important in controlling exchange processes between streams and fluvial plain groundwaters. Malard *et al.* (2002) present a landscape perspective on hydrological exchanges between the surface and subsurface that emphasizes bed topography, sediment permeability, patch size, spatial arrangement of patches, and the dynamic nature of surface-subsurface exchange pathways. Kasahara and Wondzell (2003) show pool-step sequences drive hyporheic exchange in low order streams while pool-riffle sequences, channel splits, and secondary channels are important for hyporheic exchange flow in unconstrained midorder streams. Constrained stream reaches show little hyporheic exchange flow. Cardenas *et al.* (2004) conclude that streambed topography and substrate heterogeneity are important for hyporheic zone geometry, fluxes, and residence time distributions. The fields of geomorphology, landscape ecology, and hydrogeology are providing improved understanding of hyporheic zones at the landscape scale.

In this chapter, we describe field methods for sampling the hydrology, chemistry, and biota of the hyporheic zone, with emphasis placed on understanding hyporheic zone

hydrology. A variety of levels of sophistication are presented, ranging from excavating pits in alluvial sediment to installing a permanent well field and instrumenting the wells with pressure tranducers to measure water table elevations. These field methods can be applied to the study of hyporheic zones located subchannel or lateral to streams or rivers. Finally, these protocols emphasize that access to the hyporheic zone remains one of the major challenges in studying this interface.

Throughout the rest of this chapter, we use the term groundwater to describe subsurface water that will be sampled. Much of this water is classified as hyporheic zone water, but in some locations the sampling wells may access a portion of the groundwater system that has not yet interfaced with the stream water contribution. Therefore, the more general term of groundwater will be used to identify the water samples collected from the saturated zones of stream and floodplain sediments.

Specific objectives of this chapter are to describe methods that can be used to (1) determine the direction and velocity of groundwater flow in the hyporheic zone, (2) measure vertical hydraulic gradients to characterize the direction of vertical flow between surface and subsurface waters, (3) estimate hydraulic conductivity of hyporheic zone sediments, and (4) describe sampling protocols for collecting samples for physical, chemical, and biological variables. Choice of which of the five exercises to use in the field will depend on characteristics of the stream, the availability of sampling points to access groundwater in the hyporheic zone, and the magnitude of effort appropriate for research or educational goals.

II. GENERAL DESIGN

Each of the various exercises requires a means to sample groundwater in the region adjacent to the stream or below the active channel. In all cases, a strong back and a stout heart are needed to prepare for sampling of these subsurface environments. Site selection should consider local geomorphology and stream sediment size; stream reaches with considerable bedrock exposure at the surface or dominated by large boulders are more difficult to instrument. In general, unconstrained reaches (sensu Gregory *et al.* 1991) of stream are more amenable for the described methods. Convex bedform where lower gradient segments of stream begin to steepen are generally areas of surface water recharge into groundwater (downwelling), while concave bedform where higher gradient reaches change to lower gradient sections are commonly zones of groundwater discharge (upwelling) (Vaux 1968, Thibodeaux and Boyle 1987, Harvey and Bencala 1993). A conceptual view of this channel unit interaction between bedform and hyporheic zone flowpaths is shown in Figure 6.1. An unconstrained reach of stream with a riffle-pool-riffle sequence is a good site for these procedures and, once a proper reach is identified, properties of the hyporheic zone may be investigated with the following techniques.

A. Sampling Pits

A straightforward way of sampling the hyporheic zone is to dig a hole with a shovel and crowbar into the floodplain and/or exposed channel bars near the active channel. One advantage of this procedure is that it allows an accurate determination of the height of the water table. The elevation of the top of the saturated zone determines the location of the water table at that location. Continue to dig the hole to a depth of 30–50 cm below the water table if possible. The pits may then be used to sample
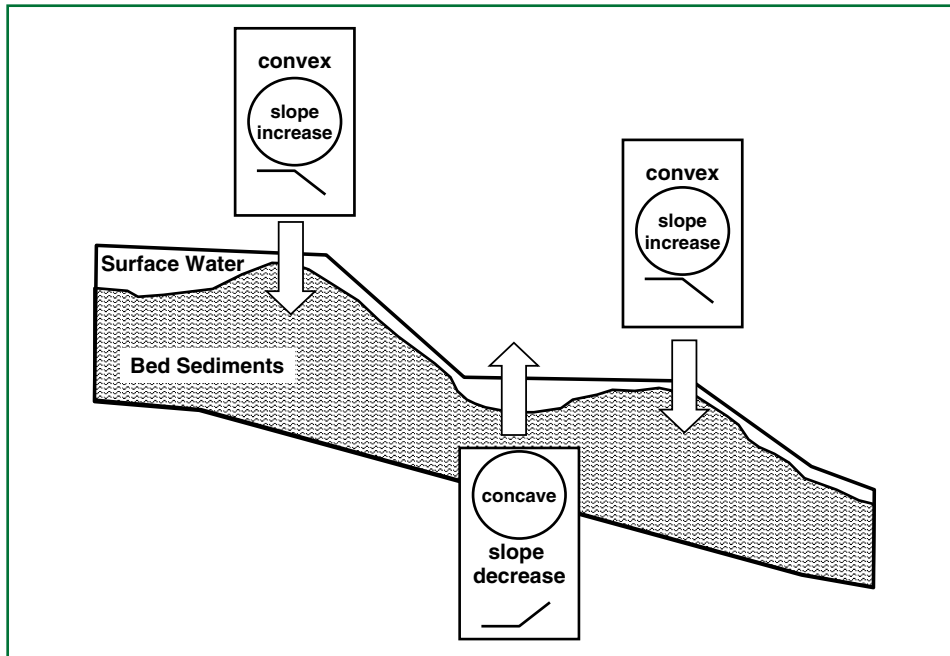
FIGURE 6.1 A schematic representation of changes in the direction of vertical hydrologic exchange (large arrows) is shown in response to alterations in streambed morphometry (after Vaux 1968, White *et al.* 1987).

hyporheic water, sediments, chemistry, and biota. These excavations also can be used to estimate groundwater velocities by adding a salt or dye tracer to the standing water. As groundwater is flowing through the pit, measurements of dye or salt concentration dilution over time (borehole dilution tests) can be performed and analyzed to compute the local groundwater velocity. If flow in the sediments is sufficiently fast, smaller holes can be excavated around the initial pit and used to record whether the tracer appears and the variation in tracer concentration with time. These data also can be analyzed to calculate local groundwater velocities (see the tracer test discussion in monitoring wells below). The distance from the main pit to the secondary sampling locations should consider the texture of the alluvium. Fine-grained alluvium calls for secondary sampling sites within 10–30 cm, whereas pits in coarse-grained alluvium may be placed 50–100 cm distant.

B. Minipiezometers

The potential for vertical exchange of channel water and groundwater can be evaluated using minipiezometers (Lee and Cherry 1978). These hollow tubes are essentially small diameter wells in which the elevation of water levels in saturated sediments can be measured. Preparation and emplacement of minipiezometers is relatively easy with proper tools. Minipiezometers often are installed by driving the tubes into the sediments using manual methods. The water level in the piezometer is then compared to the stream water level immediately outside of the tube to determine if the water level in the piezometer is higher than the stream stage (groundwater is moving into the stream) or lower (stream

water is moving into the saturated sediments). In addition, when this difference in water levels is determined and divided by the depth of the piezometer penetration into the saturated sediments, the Vertical Hydraulic Gradient (VHG) can be computed. A positive VHG indicates upwelling and a negative VHG indicates downwelling (Figure 6.2). This value, when combined with measured properties of the saturated sediments, is used to compute exchange rates.

Minipiezometer construction and installation varies depending on the available budget, sampling plan, and character of the saturated sediments. Often $1''$ diameter (2.54 cm) steel, PVC or CPVC pipe are selected, but smaller or larger diameter tubes can be used. Piezometers are most often driven into place using a conductor tubing (slightly larger in diameter) with a center removable rod or a disposable tip (Lee and Cherry, 1978). A PVC or other composition pipe with a perforated interval is then installed and the conductor casing removed. A second approach has the researcher directly drive a tube with a perforated section with either a permanent tip or a disposable tip into the sediments with that instrument becoming the minipiezometer. In all cases, the piezometer needs to be in communication with the underlying saturated sediments either by an open end or holes and slots cut into the bottom portion of the tube. Driving is most often accomplished by a sledgehammer, fence post driver, or slide hammer. Piezometers also should be developed by pumping or surging to assure that they are not plugged with sediment and are freely connected to the groundwater. Minipiezometers can be placed in transects across the active channel and into adjacent floodplain sediments, or on an upstream to downstream (longitudinal) channel transect to measure either lateral or longitudinal patterns in VHG and to sample hyporheic water quality.
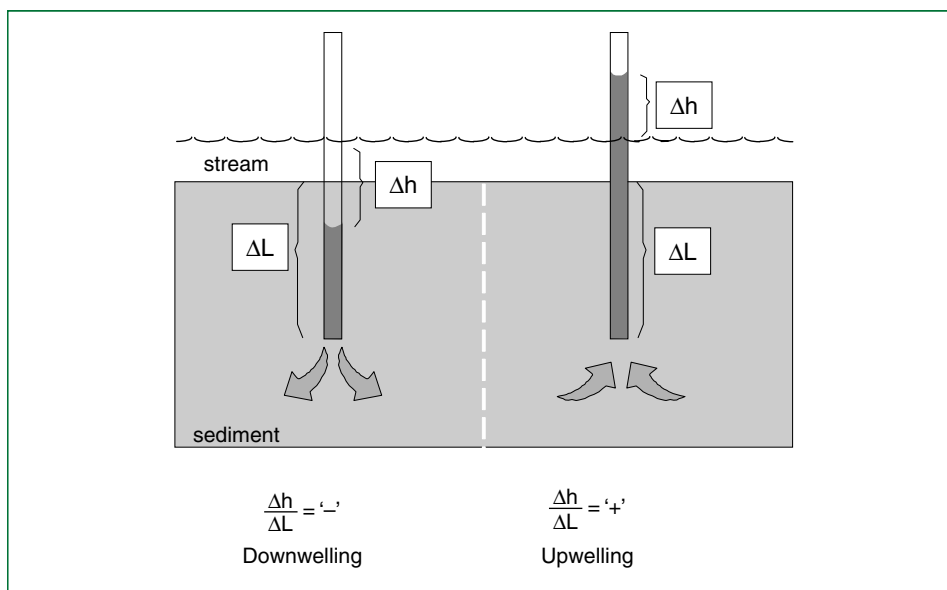

FIGURE 6.2 Vertical hydraulic gradient (VHG) in a downwelling and upwelling region of the hyporheic zone is represented as a function of Δh (difference in head between the water level in the piezometer and the level of the stream surface) and ΔL (depth from the streambed to the bottom of piezometer for a solid pipe open at the bottom). The value of ΔL would be to the midpoint of the perforations or screened section for a piezometer with a slotted or perforated design.

C. Monitoring Wells

Groundwater movement, direction and flow rates are determined from computing the position and slope of the water table and the transmission properties of the saturated sediments under investigation. The water level elevations obtained from the wells and results of hydraulic conductivity testing are combined in the basic groundwater equation (Darcy's Law) to compute discharge and groundwater velocities. Darcy's Law relates the slope of the water table (hydraulic gradient = i), the horizontal or vertical cross sectional area (A), and the transmission properties of the sediments (hydraulic conductivity = K) to the groundwater flow rate (discharge = Q):

$$Q = KiA \tag{6.1}$$

By adding the effective porosity (n) of the unconsolidated sediments (typically equal to the specific yield for unconfined aquifers, S_y), the groundwater velocity can be estimated:

$$v = Ki/n \quad \text{or} \quad v = Q/An \tag{6.2}$$

As monitoring wells are used as windows into the groundwater system, their design often attempts to allow for water level determination, water quality sampling, biological sampling and the characterization of the transmission properties of the sediments.

Monitoring wells are most commonly constructed of steel or PVC. They can be almost any diameter, however, the 51 mm diameter (2″) well is often used in groundwater investigations. These wells are often capped and perforated over a specified length above the well base. The length of the perforated interval and the number and diameter of the perforations are often designed to test a limited vertical section of the groundwater, provide unrestricted movement of water between the well and the groundwater, and yield sediment-free water samples. Though these are desirable design goals, they may need modification to accomplish research objectives (e.g., a perforation slot size that allows macroinvertebrates to enter the well may also allow sediment in the well bore during water quality sampling).

Installation of monitoring wells may be completed by hand augering or digging, hand hammering, direct push drilling (e.g., GEOPROBE), mechanical auger drilling, and a number of more sophisticated drilling techniques (forward rotary, dual tube, and rotosonic). When well installation needs to be completed by truck mounted rigs, access is limited and instream use is usually unacceptable. Fortunately, wells for hyporheic studies are often shallow, can be smaller diameters, and constructed by hand work. The length of slotted screen determines the range of depth from which water will be sampled. The location and length of the slotted portion of the well pipe (screen) can be tailored for sampling of sediment intervals. Although the initial effort of well installation may be laborious and time-consuming, wells located out of the influence of high river flows are likely to provide sampling opportunities for many years with minimal maintenance.

Once the casing material is placed in the ground, if the well diameter is smaller than the completed hole and the borehole does not collapse around the casing, the length of the well that is perforated can be surrounded by a uniform sand or gravel that is added to the borehole. The diameter of this material should be slightly larger than the perforation diameters (slot opening) so that flow into the well is not restricted. Above the perforated interval, the hole can be back filled with native material or a lower conductive material like a bentonite (clay, pellet, or granular form). The wells completed on dry land should have about 10 cm of bentonite added to the hole at the surface to prevent infiltration of surface runoff. Once a well is installed in the stream bed, efforts should be made to prevent stream water from short circuiting to the perforated interval along the well casing. This can be done by adding bentonite in the borehole at the river bottom, or more commonly by packing native sediment around the well bore at the river bottom. As described with the minipiezometers, monitoring wells should be developed by bailing, surging or pumping to assure wells are freely communicating with the groundwater before water level or other data are collected.

D. Water Level Measurement

An important aspect of understanding how hyporheic exchange occurs is revealed by observing how surface water and groundwater elevations change at an observation point and relative to each other. The position of the stream stage is easily derived by driving a steel post into the stream bed and either measuring the water stage from the top of the post or the water level shown on a metal ruler strapped to the post and partially submerged. A water level can also be obtained from a bridge by lowering a tape from a fixed point to the water surface.

Water level measurement in minipiezometers and wells can be completed manually using a steel tape or electric water level monitor (e.g., manufactured by companies like Solinst, Insitu, and Heron). The use of a steel tape requires coating a portion of the end of the tape with a chalk dust or water soluble paste or ink (e.g., Vis-a-Vis™ overhead pens). The tape is lowered into the well and held at an even foot or meter marking (hold value) so that a portion of the tape extends into the water. The tape is then withdrawn and the length of wetted tape is subtracted from the hold value. Commercial electric water level tapes use a battery-powered unit with a graduated cable and a sensor that lights a light or causes an audible signal when the probe enters the water. The measuring tape is lowered into the well and, when the signal that water has been encountered has been received, the cable is moved up and down to carefully define the level. The reading of the depth to water is then directly read from the hold point. Probes with appropriate probe diameters are needed to sample small diameter wells. Other mechanical methods to sample water levels in small diameter minipiezometer wells include using thin rods coated with a dusting of chalk that are lowered into the well and then withdrawn. The rod is either calibrated or a hand carpenter's tape is used to determine the wetted length and the distance to the water level. Modifications of this technique include outfitting the rod with wires and a circuit to create a signal when water is encountered (see Baxter *et al.* 2003).

When information about water level change over time is desired to document natural changes and responses or during a hydrologic test, the use of a water level recording device is appropriate. These can be used to record both surface water and groundwater elevations, depending on site conditions. Recording devices include float and chart

recorders, manometer and bubbler recorders, resistivity bridges, and electrical transducers. The use of each of these techniques requires either temporary or more permanent facilities. For hyporheic investigations where small diameter wells (<2″ diameter) are often used, water table elevations are commonly measured with pressure transducers. A common type of pressure transducer utilizes a strain gage transducer, which is connected to a pressure-sensing element and a data logger. The instrument measures the overlying pressure of the column of water above the submerged transducer. Two types of transducers are commonly used, those vented to the atmosphere and those that are unvented. The vented systems correct for the weight of the atmosphere and provide true water height in the well or river, while the data from the unvented systems need to have a second transducer corecording atmospheric pressure so that a post-processing correction can be executed.

Transducers can be purchased in a variety of depth of submergence ranges and accuracies. They come in cable connected and stand alone units with various diameters (many will fit in 1″ diameter wells or tubes). Numerous manufacturers exist, including Solinst Canada Ltd. (Georgetown, Ontario); In Situ, Inc. (Fort Collins, CO); Design Analysis Associates, Inc. (WaterLog series, Logan, UT); Global Water Instrumentation, Inc. (Gold River, CA); and Onset Computer Corporation (Bourne, MA). Prices are highly variable, ranging from several hundred to more than $2000, depending on the model (some can also measure conductivity), communication options, and so on. A barometric pressure logger, required at sites with unvented pressure transducers, costs several hundred U.S. dollars.

When more than relative water level changes within a network of wells are desired, all hold or water level reference points can be surveyed using standard techniques to a common local datum or the bench mark mean sea level. Once this is completed, maps of the water table position can be constructed and horizontal and vertical gradients computed (see Baxter and Hauer 2000 and Valett *et al.* 1994). Based on network maps, groundwater flow can be interpreted as occurring at right angles and down gradient to contours of equal groundwater elevation (head). The connection of the stream to the floodplain groundwater also can be evaluated when a network includes near channel wells.

III. SPECIFIC METHODS

A. Method 1: Measuring Groundwater Velocity — Pit or Borehole Dilution Method

1. This method can be used in either a hand-dug pit or a fully perforated piezometer or monitoring well. The groundwater velocity is computed from these field data.
2. In this method, we use a shallow pit constructed in a floodplain on a sand or gravel dominated point or channel bar (for use in a piezometer or monitoring well, see the description by Lamontagne *et al.* 2002). Once the pit is constructed so that it extends into the water table, the volume (V) of the water in the pit is computed by measuring the water depth and pit dimensions. An average cross-sectional area (A) is computed by using the diameter of the hole and the water depth.
3. A conductivity meter is used to establish the initial (background) concentration of water in the pit. A NaCl solution is prepared in a container filled with river or pit water. At time zero, the NaCl solution is added to the pit so that the water conductance becomes about five times that of the background concentration. As the tracer is added, the conductivity probe or another stirrer is used to gently mix

the water in the pit to a uniform concentration. This process should take less than 30 s. Once the tracer has been added and mixed, the experiment starts.

4. The conductivity meter is used to monitor the change in pit conductivity over time (gently stir the pit water prior to each measurement). A decrease in conductivity results from the inflow of naturally low background groundwater. Time and concentration are recorded in your field notebook. These data are collected until the concentration in the pit returns to background. It is recommended that the data collected during the change in concentration from 150% of the background to near background (consistent slope is observed) actually be used for computations (graph construction).

5. Once the test is completed, a plot of the relative concentration C_{t*} versus time is constructed where $C_{t*} = (C_t - C_b)/(C_0 - C_b)$, where C_t is the concentration at time t, C_b is the background concentration and C_0 is the concentration once all the tracer is added to the pit (initial concentration at time 0) (see Lamontagne *et al.* 2002).

6. Once this graph is constructed, the pit parameters are entered in the following equation: $C^* = e^{-(v^*At)/V}$ and the value v^* is estimated by trial and error substitution to generate a plot that matches the field data (observed in step 5). This process is best completed using a spreadsheet like Excel®. The parameter v^* is referred to as the apparent velocity.

7. The computed v^* (apparent velocity) is actually higher than the true groundwater velocity as the presence of the pit (removal of the sediment) enhances the groundwater velocity within the pit. As a result, the computed apparent velocity value is adjusted to an estimate of the groundwater velocity (v) as follows: $v = v^*/an$, where a is the shape factor (2 for homogeneous porous media) and n is the effective porosity (based on field determination or tables — for example, silt = 0.03 to 0.19, fine sand = 0.10 to 0.28, medium sand = 0.15 to 0.32, coarse sand = 0.20 to 0.35, gravelly sand = 0.20 to 0.35, fine gravel = 0.21 to 0.35, medium gravel = 0.13 to 0.26, and coarse gravel 0.12 to 0.26 (Fetter, 2001)). (Note the a factor may increase with less uniform sediment.)

8. Be sure to refill or cover the pits at the end of the experiment for the safety of wildlife and people visiting the stream.

B. Method 2: Groundwater Flow Direction and Velocity Using Tracer Injection and a Network of Down Gradient Monitoring Pits or Wells

1. This method makes use of hand-dug sampling pits, a network of minipiezometers, or a well field. The direction and velocity of groundwater flow will be measured.

2. The placement of observation pits, piezometers, or wells near the injection point will influence the success of this field test. This is based on the anticipated velocity of groundwater flow in different types of material, and the location of the sampling points in the groundwater flow system relative to the injection point. In fine-grained sediments, observation points will need to be placed close to the injection site and open to the same interval of saturated sediment. Average velocities will be in the range of cm d^{-1} in silts, 10's of cm to a meter per day in sands, and meters to 10 meters per day in sand and gravel. Sampling locations should be spread out laterally in a half circle in the perceived direction of the water table slope. If more extensive water table elevation data are available for the study area, the slope of the water table can be mapped from contouring water table elevations normalized to a local or regional datum. The general direction of groundwater flow is assumed to be perpendicular to the contoured surface.

3. Prior to the beginning of the experiment, establish background concentrations of the tracer being used. Start the test by either injecting a distinctly visible dye such as fluorescein or rhodamine WT or a salt tracer into a center well or pit and record the start time of the injection and the initial concentration. Pulse injections can be used where all dye is injected at one point in time, or a continuous long-term injection may be employed using a metering pump (keeping the input rate and concentration constant) if proper equipment is available.

4. Sample nearby wells, minipiezometers, or pits for appearance of the dye or changes in conductivity. Use a field conductivity meter if a salt tracer is being used. A portable field fluorometer can be used to measure the concentration of the dye if a fluorescent dye tracer is used. If sampling is done by extracting a volume of water, this should be a small volume so that the groundwater flow system is not disrupted by the sampling (samples of 10 to 20 mL are suggested). If using a conductivity meter, place the probe at the same observation point for each measurement. The application of visually detectable dyes works well for relatively short transport distances and times. Dye samples collected from the sampling points should be stored in clear containers and stored out of the sunlight if fluorescein is used. If a slug input of tracer was used, the conductivity data can be plotted versus time and the dye samples lined up in order of sampling time so that the time corresponding with the peak concentration of dye or conductivity can be estimated. This peak time is then used to compute groundwater velocity by dividing the peak time into the distance between the sampling point and the injection site; v = distance of travel/time of peak concentration arrival. If a continuous source injection was used, the time of arrival of 50% of the relative concentration C/C_0 (where C is the measured concentration at time t after the test begins and C_0 is the concentration of the tracer injected) is the time selected for the velocity calculation. The observation of the first appearance of the tracer at a site represents the most rapid transport the tracer undergoes (within the detection limits to observe the tracer). The peak arrival time during the slug injection or 50% of C/C_0 for a continuous injection are used to represent the average transport rate of the groundwater.

5. Arrival of the tracer in observation pits or wells also can be used to estimate the local direction of groundwater flow in the study area. Measurements repeated under changing conditions of floodplain and channel flow can determine the variability in groundwater flowpaths as the hydrograph varies.

6. If pits are used to determine the direction and velocity of groundwater flow, be sure to refill or cover the pits at the end of the experiment for the safety of wildlife and people visiting the stream.

C. Method 3: Measuring VHG in Minipiezometers

1. Carry out the following measurements on all minipiezometers within the study reach. Measuring the height of the water within the minipiezometer and the relative river stage is easily accomplished using a variety of techniques as describe in the previous general water level measurement section. The selection of a specific technique is dependent on the minipiezometer design (length and diameter) and the challenges of working in the stream channel (flow velocity and stream depth).

2. Design and installation of the minipiezometer will depend on project objectives and available materials and equipment. This method reported by Baxter *et al.* (2003) is used to install ½ inch diameter minipiezometers in a gravel dominated stream bed.

Their installation used an outer sleeve or casing, a pointed driver rod that fits inside the casing, a PVC minipiezometer, and a hammer cap that fits over the top of the driver (Figure 6.3). A sledgehammer and channel grips or pipe wrenches also were
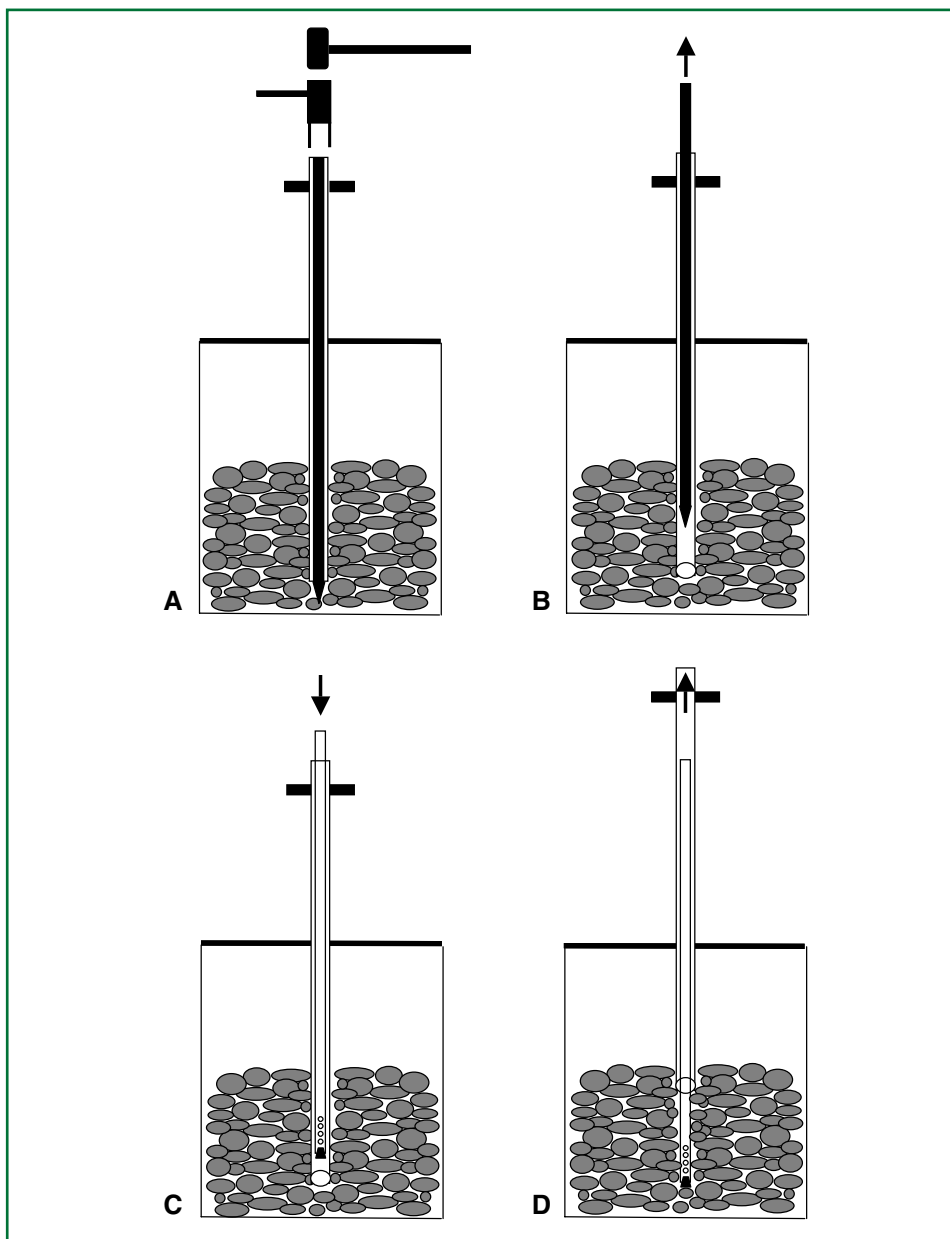

FIGURE 6.3 Sequence of procedures for installation of a minipiezometer. (A) Driver mechanism with a hammer cap is driven into streambed. (B) Steel driving rod is removed with casing held in place. (C) Minipiezometer is slipped inside casing. (D) Minipiezometer is held in place while steel casing is removed. The minipiezometer is ready to be developed for sampling (from Baxter *et al.* 2003, used by permission).

needed for installation. The outer sleeve or casing design can be modified, depending on site conditions. Baxter *et al.* (2003) used a ¾ inch (19 mm) diameter stainless steel pipe with a stainless steel collar ring welded 2 inches (51 mm) below the top. The driver rod (solid cold-roll steel) should fit snugly inside the casing with a machined point on the insertion end. The outer casing needed to be filed down where the point of the driver rod protrudes so that the lip will not hang up during installation. Installation proceeds as follows (Figure 6.3): (1) the driver mechanism (casing with driver rod inserted) is placed on the stream bottom and the hammer cap placed on top of the collar; (2) this apparatus is then hammered into the streambed to the desired depth (marked on the outside of the outer steel casing) with the sledgehammer; (3) the steel driving rod is removed using vice grips so that the imbedded casing remains in place; (4) the minipiezometer is then slipped inside the casing; (5) then, holding the minipiezometer in place using a short piece of pipe, the steel casing is removed using the vice grips; and (6) stream sediment is tamped around the minipiezometer to prevent direct flow along the casing to the perforated or slotted interval (Baxter *et al.* 2003).

3. The minipiezometer now needs to be developed to ensure that the perforated or slotted interval is communicating with the surrounding groundwater. This can be accomplished by taking water out of the piezometer or putting water into the piezometer and determining if the water level returns to a static stable level. This may be a slow process in fine-grained sediments and may require that you wait hours before checking for refilling. This procedure may need to be repeated multiple times to assure that there is good connectivity between the minipiezometer and surrounding groundwater.

4. For minipiezometers installed in the active channel, measure the distance from the top of the minipiezometer to the stream surface. This is best done by attaching a "stilling well" to the minipiezometer. A hollow tube (same diameter as the minipiezometer) is attached to the side of the minipiezometer with plastic clips. Place the stilling well alongside the minipiezometer in a line perpendicular to stream flow with the stilling well bottom near the substrate and the top extending well above the stream surface. This allows determination of the surface elevation of the stream more accurately as water run-up and downstream eddies are avoided (Figure 6.4).

5. Measure the water level inside of the piezometer using the top of the well as a hold or reference point. Measurement techniques are presented in the preceding section. Repeat steps 4 and 5 for all minipiezometers at the study site. Record the depth to which the minipiezometer is installed into the river bed (L) at each site. This is computed as the distance from the stream or river bottom to the top or middle of the perforated interval.

6. For minipiezometers in the stream, calculate VHG for each minipiezometer

$$\text{VHG} = \frac{h_s - h_p}{L} \tag{6.3}$$

where h_s = distance from the top of the minipiezometer to the stream surface, h_p = distance from the top of the minipiezometer to the water level inside the pipe, and L = depth of minipiezometer into the sediment. The resulting
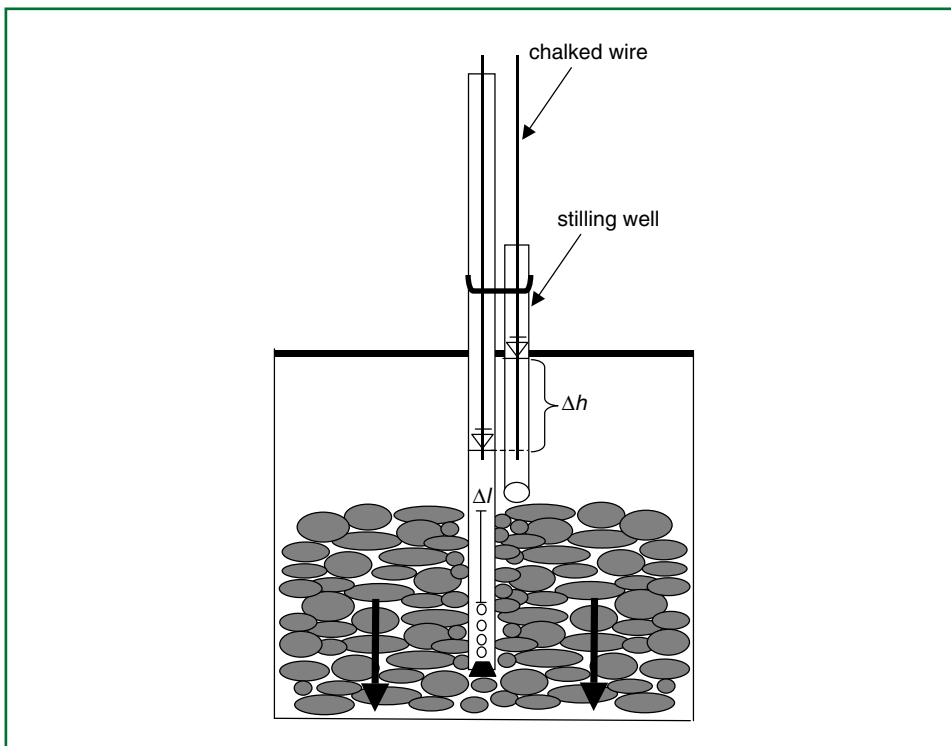
FIGURE 6.4 Minipiezometer and stilling well with chalked wire used for measuring water levels inside the minipiezometer and determining the surface stream water level (from Baxter *et al.* 2003, used by permission).

unitless ratio will be positive in upwelling (groundwater discharge) and negative in downwelling (groundwater recharge) zones.

7. Map out the pattern of upwelling and downwelling zones in the study section of stream. An example of such a two-dimensional map is shown in Figure 6.5. The upper panel of the map shows the streambed and surface water elevations, and the lower panel shows regions of upwelling and downwelling as measured by VHG (cm/cm). A three-dimensional map can be constructed if the piezometer/well locations are surveyed. Contours can be generated for VHG by applying an algorithm and plotting using mapping software such as Surfer® (see Valett *et al.* 1994 and Baxter and Hauer 2000).

D. Method 4: Measuring Horizontal Hydraulic Conductivity (K_h)

1. This method is designed for minipiezometers or wells and explains how to estimate horizontal hydraulic conductivity (K_h) in saturated sediments. The slug test method is based on instantaneously making the water level in the instrument either rise or fall, and then recording the behavior of the water level versus time as the water returns to its original position. These data are collected in the field along with
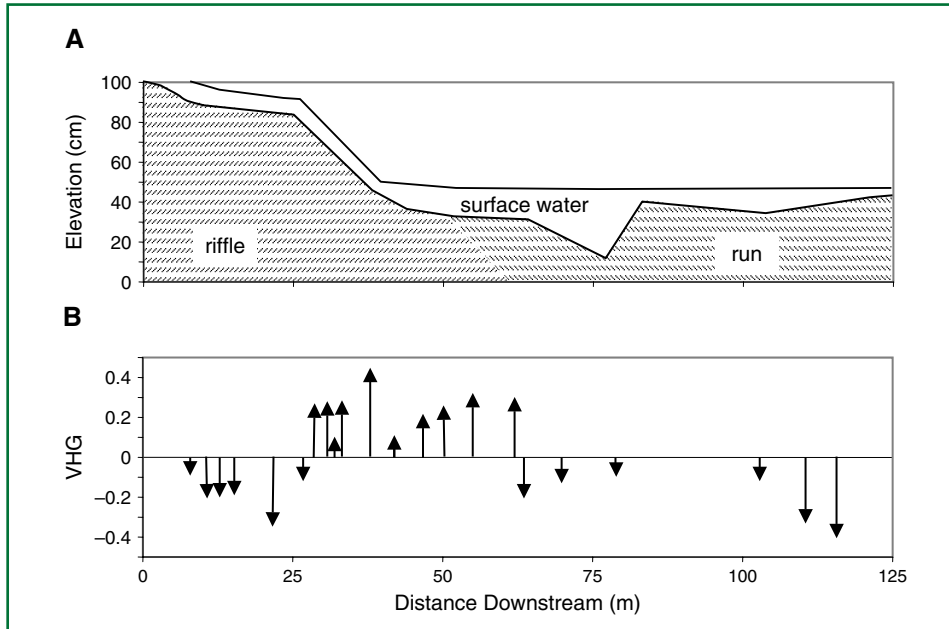
FIGURE 6.5 (A) Longitudinal section of a 125-m study reach at Sycamore Creek, Arizona, illustrating downstream changes in streambed elevation and morphometry. Riffles are steeper gradient sections where substrata are cobbles/boulders. Runs are lower gradient reaches where substrata are predominantly sand and gravel. (B) Magnitude of vertical hydraulic gradient (VHG) (cm/cm) along a midstream longitudinal axis of the same study reach.

information concerning well construction. Water level changes in minipiezometers or monitoring wells can be induced using a number of methods: (1) a small bailer is used to physically remove a volume of water or water is rapidly poured into the well; (2) a solid weighted slug that is a smaller diameter than the well bore is released into the well displacing the water and causing the water level to rise (or decline if the weighted slug is pulled out of the water filled well bore); and (3) the well is closed off and outfitted with an airtight valve with compressed air so that as air pressure is increased the water level is depressed and a water level rise occurs when pressure is quickly released. In each of these methods, the initial depressed or raised water level position and the following water level response need to be recorded once the test begins. This requires either manual measurements of water level change (see the water level measurement section above) or the use of a recording electrical transducer. The selected method depends upon how quickly water levels change in the well bore. A test in silty sand may last for minutes to hours while a test in coarse sand or sand and gravel may be over in less than one minute.

2. The three most widely applied techniques for estimating K_h are the Hvorslev (1951), Bouwer and Rice (Bouwer and Rice 1976, Bouwer 1989), and Butler's modification of the Bouwer and Rice approach (Butler and Healey 1998, Butler *et al.* 2003). Other modifications of these techniques also are reported in the literature. The Hvorslev (1951) equation and analyses is relatively easy to use. The form presented

here is applicable for a well with a perforated or slotted (screened) length (L_p) that is more than eight times the radius of the slotted casing (R) ($L_p/R>8$) (Freeze and Cherry 1979). For example, with a 16 mm diameter minipiezometer, the length of perforated or screened well should be at least 128 mm. This set of conditions results in the following equation:

$$K_h = \frac{(r^2) \log_e \left(L_p/R\right)}{2L_p T_0}$$

(6.4)

where K_h = horizontal hydraulic conductivity (cm/sec), r = radius of minipiezometer or well casing (cm), R = radius of the perforated or slotted screen interval (cm), L_p = length of perforated or slotted screen interval (cm), and T_0 = basic time lag for water level to return to 37% of initial change in water level. The basic time lag (T_0) is determined when water is either added to or removed from the minipiezometer or well. The water level change through time after water addition or removal is normalized for the maximum water-level change and plotted on a log scale versus time. Compute the ratio h/h$_0$, where h$_0$ is the height the water level either dropped to if the static water level was lowered or rose to if the water level was raised. The value of h is the water level drop or gain at some time t after the initial water level change. Plot the ratio h/h$_0$ versus time on a semi-log basis using time as a linear variable; the data should plot on a straight line. T_0 in units of time is the corresponding normalized water level change equal to 0.37 (Freeze and Cherry 1979, Fetter 2001). The Bouwer and Rice (1976) method and its reported modifications also can be used to interpret more complex geologic settings and should be evaluated depending on site conditions.

3. Butler *et al.* (2000) present a spreadsheet method to analyze slug test responses in highly conductive materials collected using a rapidly recording transducer. This work uses a modification of the Bouwer and Rice (1976) analyses. The following site describes the analyses and a spreadsheet method for the calculation (http://www.kgs.ku.edu/Hydro/Publications/OFR00_40/index.html). Baxter *et al.* (2003) also attempted to estimate values of K_h from slug tests in highly conductive stream sediments, where the elevated water level in the minipiezometer fell at such a high rate that manual water level changes could not be obtained. For these highly conductive sediments, they derived a relationship that correlated well with test results in the same sediments, where more complete water level and time data were available. The information that was recorded in these areas of rapid water level change was the well construction and the time it took for the water to fall from an elevated position to the static water level. They estimated K_h as

$$K_h = \left[\frac{L_s (0.7854) \left(d_{piezometer}\right)^2}{\pi (d_{perforated\ interval}) L_p \Delta t} \right] \left[\log_e \frac{h_0}{h} \right]$$

(6.5)

where h_0 is the starting head in the piezometer (raised level) and h is the head in the piezometer at a single time (Δt), L_s is the length of the horizontal path the slug impacts (travels into or from) the aquifer, L_p is the length of the screen, and Δt is the time it takes to go from initial (h_0) to static water level (h). When L_s is assumed equal to L_p and the diameter of the piezometer and screened interval are equal, Baxter *et al.* (2003) showed that for their well design ($L_p/R > 8$ where R is the radius of the well screen) K_h would be reasonably estimated.

4. Measurements of K_h also allow estimation of vertical hydraulic fluxes. Assuming that the values of vertical hydraulic conductivity (K_v) are approximately 0.10 of K_h (Anderson and Woessner 1992), one can estimate the vertical component of water flux in the streambed near the piezometer or well. Specific discharge (q; $cm^3 \cdot cm^{-2} \cdot s^{-1}$) is calculated by the equation

$$q = K_v \left(\frac{\Delta h}{\Delta l} \right) \tag{6.6}$$

where $\Delta h/\Delta l$ is the VHG. As in the case for VHG, q can be mapped (see Baxter and Hauer 2000, for example).

E. Method 5: Sampling a Well Field

1. This exercise is designed for those sites where a well field has been installed. A survey map with well locations and elevations is required for the exercise.
2. Measure the height of the water table in each well. Be sure to also measure the stage height of the stream at various locations within the well field. A map and contours of the groundwater system can be constructed if each well and surface water stage measurement has been surveyed relative to the same horizontal datum.
3. Bail all the wells and allow them to recharge. After recharging, the wells can be sampled for temperature, conductivity, pH, and dissolved oxygen. Measurements are best made with portable field probes inserted into the groundwater within the wells. If field probes are not available, samples can be drawn into large gas-tight syringes (60 mL) with Tygon extensions and measurements made using standard methods (e.g., APHA *et al.* 1992). The number of physical and chemical measurements should be guided by available instrumentation and sampling objectives.
4. Chemical and invertebrate sampling can also be carried out using groundwater wells. Bailers, syringes, or peristaltic pumps can be used to collect samples for chemical analyses. Standard methods for sample preparation and storage should be employed after samples are withdrawn (APHA *et al.* 1992). Special care needs to be given to avoid contact with the atmosphere for samples collected for gas analyses. Sampling for invertebrates requires unscreened wells with open bottoms and sidewall slots, a high-volume diaphragm water pump, and a fine-meshed net (e.g., 45 μm plankton net) to collect the organisms exiting the pump. Sampling methods for hyporheic zone invertebrates from wells are presented in more detail by Stanford

and Ward (1988), Hakenkamp and Palmer (1992), Dole-Olivier *et al.* (1997), Boulton *et al.* (1997), Malard and Hervant (1999), Malard *et al.* (2003a), Malard *et al.* (2003b), Olson and Townsend (2003), and Olson and Townsend (2005).

IV. QUESTIONS

1. What is the direction of flow for groundwater at your sampling site? What is the groundwater velocity? How does the direction of flow and velocity change as stream discharge changes?
2. What is the pattern of VHG values for the minipiezometers beneath your stream? Is this section of channel gaining or losing surface water due to exchanges with groundwater? Do the locations for upwelling (groundwater discharge) and downwelling (groundwater recharge) conform to the predicted relationship with bedform concavity and convexity?
3. How does VHG change with increasing stream discharge? What happens to the elevation of the water table along the edges of the channel?
4. Are there measurable changes in water level in groundwater of the hyporheic zone during a diel cycle with no precipitation during that period of time?
5. Darcy's Law can be used to calculate the vertical flux of groundwater (volume per unit time) with the following equation:

$$Q = KAi \qquad (6.7)$$

where Q = flux of groundwater (m³/day), K = hydraulic conductivity (m/day), A = area through which flow occurs (m²), and i = vertical hydraulic gradient (VHG, unitless). Given an area of 1 m², VHG measured with your minipiezometers, and K_v estimated as 10% of K_h measured by the Hvorslev method or the other methods described in this chapter, calculate the vertical flux of groundwater through the sediment-water interface of your stream. How does this value compare to total surface water discharge through the reach of stream?
6. Calculate K_h using the Hvorslev method (equation 6.2) for a well or minipiezometer at your site. Use equation 6.4 to calculate K_h for the same well or minipiezometer. How well do the two methods compare?
7. Are the groundwaters of the hyporheic zone supersaturated, saturated, or undersaturated with dissolved oxygen? Why?
8. Are the groundwaters of the hyporheic zone of lower or higher pH than the surface stream waters? Why?

V. MATERIALS AND SUPPLIES

Installation of Sampling Pits, Minipiezometers, or Wells

> 1/2″ or 5/8″ perforated schedule 40 CPVC cut to required length (for minipiezometers)

2″ schedule 40 CPVC, 2″ slotted screen or perforated pipe, slip=slip coupler, caps (for wells)

3/4″ stainless steel pipe with stainless steel collar ring

Driver rod

Hammer cap

Shovels, crowbar, channel locks or pipe wrenches, work gloves

Sledgehammer (~10 lbs or 4.5 kg)

Large volume syringe with Tygon tubing extender

Bentonite and silica sand

Sampling

Bailer that fits within size of installed wells

Biodegradable dyes

Pitcher (4 L or larger)

Sample preservatives/filtration apparatus

Sampling bottles and nets

Stopwatch

Syringe sampler

Tape measure

Water level reader (e.g., chalked dowel, chalked wire, or commercial water level reader)

Water-soluble pen

Temperature, conductivity, pH, and dissolved O_2 portable field probes

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Section B

Material Transport, Uptake, and Storage

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Fluvial Geomorphic Processes

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I. INTRODUCTION

An integral component of stream ecosystem structure and function is the relationship between flow dynamics and the movement of substratum material. Indeed, much of what we see along stream and river landscapes is a function of fluvial geomorphic processes driven by threshold entrainment of unconsolidated material by fluid flow occurring regularly over extended lengths of time coupled to brief periods of intense geomorphic work occurring during flood events. Hence, there is a landscape scale geomorphic threshold that when crossed during floods produces the physical setting, typography, and geomorphic composition of sediment deposits that shape riparian corridors and floodplains. Not only do fluvial geomorphic processes shape the riverscape (Stanford *et al.* 2005, and see Chapter 1), but also high flow velocity and scour of bed sediments often greatly affect benthic organisms (Stevenson 1990, Death and Winterbourn 1995, Townsend *et al.* 1997, Arscott *et al.* 2005). The processes of cut and fill alluviation, associated scour of bed material, and the formation of fluvially derived habitats also increase connections between aquatic and terrestrial organisms (Paetzold *et al.* 2005).

We refer the reader to Chapter 1 for an overview of landscape and riverscape processes at large scales, Chapter 2 for habitat structure commonly encountered in channels, and Chapter 4 for an introduction to the concepts of fluid dynamics in stream systems. For an exhaustive review of flow competence and streambed stability, see Lorang and Hauer (2003). The focus of this chapter is directed toward the fluvial processes associated with floodplains because floodplain systems are among the most geomorphically active and endangered landscapes worldwide (Tockner and Stanford 2002). Furthermore, it is within floodplains that the interaction between fluvial processes and geomorphic responses are most ecologically revealing.

A. Floodplain Processes and the Shifting Habitat Mosaic

Stream and river floodplains are geomorphically complex, composed of a three-dimensional mosaic of habitats of fluvial origin distributed above and below ground. We refer the reader here to Chapter 1 for an overview of the floodplain catena. In short, floodplains are composed of an array of channel habitats (e.g., riffles, runs, pools; also see Chapter 2), various riparian habitats (e.g., forest, shrub, and wetland complexes; also see Chapter 31), subsurface habitats of low and high hydraulic conductance of groundwater (e.g., the hyporheic zone; see also Chapter 6) and areas of exchange between surface waters and groundwaters (see Chapter 33). The periodic entrainment of unconsolidated material by fluid flow produces a turnover of habitat that occurs at specific rates dependent on flood flow characteristics of the watershed (see Chapter 3) and the geomorphic structure (e.g., valley configuration, size of bed material, incorporation of large wood). Geomorphic work performed during flood events coupled to riparian regeneration and plant succession provides the dynamics of geomorphic processes that leads to a shifting habitat mosaic (see Hauer *et al.* 2003, Lorang *et al.* 2005, and Stanford *et al.* 2005).

The area of the floodplain that is frequently scoured at near bank full stage levels is characterized by stream channels, backwaters, ponds, and bar formations exposed during base flow (Table 1.1). These parafluvial zone habitats are dominated by scour, erosion, and bedload deposition (Hauer and Lorang 2004, Stanford *et al.* 2005). Landforms composing the parafluvial floodplain (e.g., channels, scour holes, ponds, backwaters, spring brooks, bars, levees, banks and benches) evolve from a balance between, the available stream power (see Chapter 4) and the sediment regime composed of the sediment supply and distribution of available particle sizes. Bank and bed erosion, sediment transport, and deposition are referred to collectively as processes of cut-and-fill alluviation and are all a function of the amount of work performed by the flow of water through the riverscape or floodplain landscape (Figure 7.1).

That area of the floodplain dominated by advanced-stage regeneration (e.g., cottonwood pole stands) and mature-stage plant succession is referred to as the orthofluvial zone (Figure 7.1). An important distinction between the parafluvial and orthofluvial zones is driven by process, in that the orthofluvial lacks wide spread scouring flows. Thus, rather than rapid and repeated habitat shifts resulting from frequent scour, habitat shifts in the orthofluvial are dominated by plant succession and connection to both surface water and hyporheic waters (also see Chapter 33). The orthofluvial zone can be further divided into active versus passive areas (Figure 7.1). The active orthofluvial zone is often inundated by annual floodwaters. The riparian forest introduces high flow resistance resulting in rapid deposition of suspended sediment. The buildup of sand and finer sediment on gravel bars formed in the parafluvial is continued during flooding events in the active orthofluvial zone resulting in the formation of higher elevation shelves of recent origin (Stanford *et al.* 2005). A passive orthofluvial zone results from years of buildup of sediment and organic material, forming high shelves that eventually become inundated less frequently than the active zone. This in turn results in a much slower rate of accretion from floodwater deposition. The passive orthofluvial zone is often dominated by late successional riparian forests that exist on ancient (>1000 yr) floodplain benches (Figure 7.1). Unlike terraces, these orthofluvial benches flood frequently though without sufficient power to result in erosion. Both zones of the orthofluvial usually contain remnant channels formed when the area was occupied by the main channel and its associated parafluvial as the river channel has moved over long periods from one side of the floodplain edge to the other. The remnant channels lacing

FIGURE 7.1 An oblique aerial photograph showing the lateral extent of both the parafluvial and orthofluvial zones of the Nyack floodplain Middle Fork of the Flathead River, Montana. Location of aerial photo time-series presented in Figure 7.7 is marked by the red arrow.

through the orthofluvial can act as flood channels, spring brooks, backwaters, ponds, or paleochannel wetlands.

B. Cut and Fill Alluviation and Avulsion

Dominant orthofluvial channels are generally remnant channels resulting from past avulsion events. Channel avulsion is the process where the river, either partially or completely,

abandons an existing channel forming a new one. Channel avulsions can occur in the parafluvial or orthofluvial zones of a floodplain. Processes of cut-and-fill alluviation and channel avulsion result in a common suite of landforms (e.g., bars, channels, scour holes, ponds, and backwaters) that typify the parafluvial zone. The parafluvial can also be further delineated into active and passive subzones based on the available level of stream power. Active zones are dominated by scour, erosion, and deposition of bedload sediment in the form of bars and islands, while passive zones are dominated by the deposition of fine grained sediment from the suspension load of the river (e.g., fine sand, silt and clay). However, all types of landscape features found on a floodplain are originally formed in the active portion of the parafluvial zone. Transition from parafluvial habitats to orthofluvial habitats occurs as the dominance in fluvial process shifts from erosion, scour, and bedload deposition to deposition of suspended load, regeneration of riparian vegetation and succession (Stanford *et al.* 2005).

During floods it is common for both channel avulsion and deep scour holes to form, sometimes in association with large wood or other flow obstruction. When a scour hole or abandoned channel intercepts the underlying water table, a parafluvial pond or springbrook is formed (Figure 7.2A and B). These types of parafluvial water bodies have

FIGURE 7.2 A. Abandoned channel following parafluvial avulsion. Note remnant parafluvial pond. B. Parafluvial pond located in scour hole following bank full discharge. Note deposition of bedload forming high elevation gravel bars and sand drape formed by winnowing of fine sediments during the falling stage of the flood. C. Large parafluvial pond connected to main channel backwaters. D. Small parafluvial pond in scour hole. Note that photos A and B are in the more active zone of the parafluvial where scour and bedload deposition dominate and that C and D are in the more passive zone dominated by deposition of suspended load sands and silts a process shift that pushes the parafluvial towards orthofluvial condition.

recently been found to support a high degree of biodiversity (Chilcote 2004, Arscott *et al.* 2005, Stanford *et al.* 2005, Tockner *et al.* 2006). It is also common for floods to cause rapid and excessive deposition of bedload sediment, forming high-elevation gravel bars and islands. Often as flood waters recede, sand and fine sediment is winnowed from the exposed flat bar tops, leaving an armored layer of lag material with sand and finer sediment deposited as distinct sediment drapes along the margins of the bars, abandoned channels, and scour holes (Figure 7.2C and D). Large wood can also play a role deflecting and reducing flow velocity patterns, resulting in complex patches of surface sediments and related patches of colonizing riparian vegetation.

The physical evolution from active parafluvial to passive orthofluvial begins with either a channel avulsion event(s) or the deposition of a gravel bar(s) that moves or deflects away the erosive and scouring action of the river. When this occurs, regeneration of riparian vegetation can dominate the site as the main habitat forming process (Figure 7.2C and D). When the active portion of the parafluvial zone is moved away from newly formed geomorphic features (e.g., bars, levees, channels, scour holes and ponds), then suspended sediment deposition begins to dominate the landscape forming processes.

This shift in process dominance changes the trajectory of habitat evolution from active parafluvial to passive parafluvial and eventually to an orthofluvial environment dominated by plant succession and suspended sediment deposition (see Table 1.1). The final stage in geomorphic evolution occurs when the deposition of sediment and organic material builds the elevation of what was a parafluvial gravel bar and evolves into a passive orthofluvial floodplain bench that flood waters rarely inundate. This process can be accelerated by flow regulation aimed at flood control or from modifying channel banks with levees or rip-rap. Flood control and hydropower regulation of rivers ultimately reduces the amount of active parafluvial zone and a loss in the production of new gravel surfaces and loss of the dynamics that sustain the shifting habitat mosaic (Stanford *et al.* 1996).

Channel avulsion is a particularly important process for maintaining and renewing floodplain habitat. For example, channel avulsion can maintain an active orthofluvial zone characterized by perennially flowing springbrooks and flood channels with gravel bars that have evolved into benches and often support old growth riparian forests (Figure 7.3). These areas are dominated by overbank flooding, associated deposition of fine sediments carried as a suspension load, and the buildup of organic material. Backwater habitats connected to the main channel at the lower end of bars are a product of channel avulsion coupled to bar and island formation. Therefore, channel avulsion can result in a complex array of habitats within the active orthofluvial zone, depending on what types of cut-and-fill alluviation processes dominate through time and the relative strength of connection to the underlying groundwater table. For example, an orthofluvial springbrook that acts as a seasonal flood channel can result from an avulsion event if a natural levee on the upstream end of the avulsed channel does not form to block the inflow of flood waters (Figure 7.3A and B). Conversely, multiple avulsions or levee formation can keep an avulsed channel from acting as a flood channel, except during the very highest floods. When such orthofluvial channels are coupled to strong connections with the underlying groundwater, a perennial springbrook can form (Figure 7.3B). Conversely, complex wetland habitats can form in similar orthofluvial avulsion channels that do not act as seasonal flood channels but have strong groundwater connections coupled with frequent sediment deposition from backwater and overbank flooding (Figure 7.3C). When deposition from overbank and backwater filling processes dominate, the orthofluvial channels can attain a complete vegetative cover (Figure 7.3D).

FIGURE 7.3 A. Floodplain springbrook flowing through ancient flood channel. Note old growth forest on both sides of channel. B. Large floodplain springbrook flowing through the 1940s-era main channel. Following an orthofluvial avulsion, the parafluvial zone moved approximately 400 m, right of this photo. C. Old orthofluvial pond occupying ancient channel on the floodplain. D. Remnant channel, once active in the parafluvial zone of the floodplain, now in the orthofluvial.

C. Summary

Physical processes of cut-and-fill alluviation constantly create new surfaces for plant succession and are supplied by material that either comes from sources above the floodplain or by eroding and reworking existing surfaces within the floodplain. Patterns of sediment deposition in the form of bars, coupled to the discharge regime and the patterns of surface and groundwater exchange, control the recruitment success and establishment of riparian vegetation (Rood and Mahoney 1990). The production of large wood delivered from an eroding bank affects flow hydraulics that change patterns of sediment transport and storage, resulting in a complex feedback loop of habitat destruction and creation. Therefore, the biophysical landscape of a river floodplain results from (1) fluvial geomorphic processes that erode, transport, and deposit material; (2) patterns of surface water and groundwater connection and exchange across the landscape mediated by hydraulic conductivity (see Chapter 6) of the bed sediments vertically and laterally; (3) the distribution of water temperature; (4) the biogeochemical processes that occur in the hyporheic waters that flow into, through, and out of the subsurface lattice of fluvially sorted and deposited sediments; (5) successful establishment and regeneration of riparian vegetation; and (6) the input of elements of flow resistance, such as wood debris, coming from both turnover of riparian forests within the floodplain and from upland sources.

   This chapter has two objectives: (1) establish a descriptive link between the array of observable floodplain habitats with recognizable patterns of fluvial geomorphic processes, and (2) introduce a quantitative approach to the concept of geomorphic thresholds from a coupled geomorphic and flow competence perspective.

II. GENERAL DESIGN

A. Patterns of Process Fluvial Geomorphology

Natural rivers have long been characterized by a set of fluvial patterns including straight, braided, wandering, and meandering channels and with attempts to relate those patterns to some element of flow and sediment transport (Lane 1955, Leopold and Wolman 1957, Schumm 1963 and 1977, Kellerhals *et al.* 1976, Richards 1982, Osterkamp *et al.* 1983, Montgomery and Buffington 1993, Rosgen 1994). More recently, stream ecologists have begun to classify rivers based on habitat suitability for various organisms (Southwood 1977, Platts 1980, Bisson *et al.* 1981 and 1988, Frissell *et al.* 1986, Hawkins *et al.* 1993, Stanford 1998, Ward *et al.* 2002a and b, Weins 2002). This has lead to ecosystems being increasingly viewed as dynamic riverscapes composed of a complex array or mosaic of ever-changing habitat patches and conditions such as temperature, nutrient concentrations, and hydrologic connectivity (Fausch *et al.* 2002, Poole 2002, van der Nat *et al.* 2002 and 2003, Hauer *et al.* 2003, Malard *et al.* 2003 and 2006, Gurnell *et al.* 2005, Stanford *et al.* 2005). This mosaic of habitat changes spatially over time in response primarily to processes of channel avulsion, cut and fill alluviation, the role of wood as a flow resistance and flow deflection mechanism, and the recruitment and regeneration of riparian vegetation.

   In this chapter we group fluvial geomorphologic pattern into four basic process categories based on the typological pattern of surface water (Figure 7.4). Broad process grouping of typologies in this manner allows one to make interpretations about what types of processes are actively shaping the habitat mosaic within a specific floodplain. The ability to recognize pattern and interpret fluvial geomorphic process is important for understanding the driving forces and mechanisms that strongly affect biogeochemical processes, habitat development and succession, organism distribution and abundance, food web complexities, and production rates (Ward and Stanford 1983, Stanford and Ward 1993, Ward *et al.* 2001, Stanford *et al.* 2005). Moreover, these process regimes can be used to build the typologies of most, if not all, river systems seen across the globe.

Braiding

   Channel braiding is a process that occurs when capacity of the flow to carry sediment is exceeded by the volume of sediment being transported as bedload (Mackin 1956). This results in rapid deposition of sediment in the channel, which forces a single channel to split into two separate channels. Conceptually, this is a form of channel avulsion that results in a multitude of short-lived channels and bars and a lack of any recognizable or stable main channel. This type of river plan-form morphology is referred to as a braided river. River braiding (Figure 7.4A) represents one end of the spectrum of channel avulsion processes and results in frequent geomorphic change. Therefore, braided rivers experience rapid turnover rates of floodplain habitats and the related abiotic and the biotic drivers (Tockner *et al.* 2006). The rapid rate of habitat turnover due to braiding can result
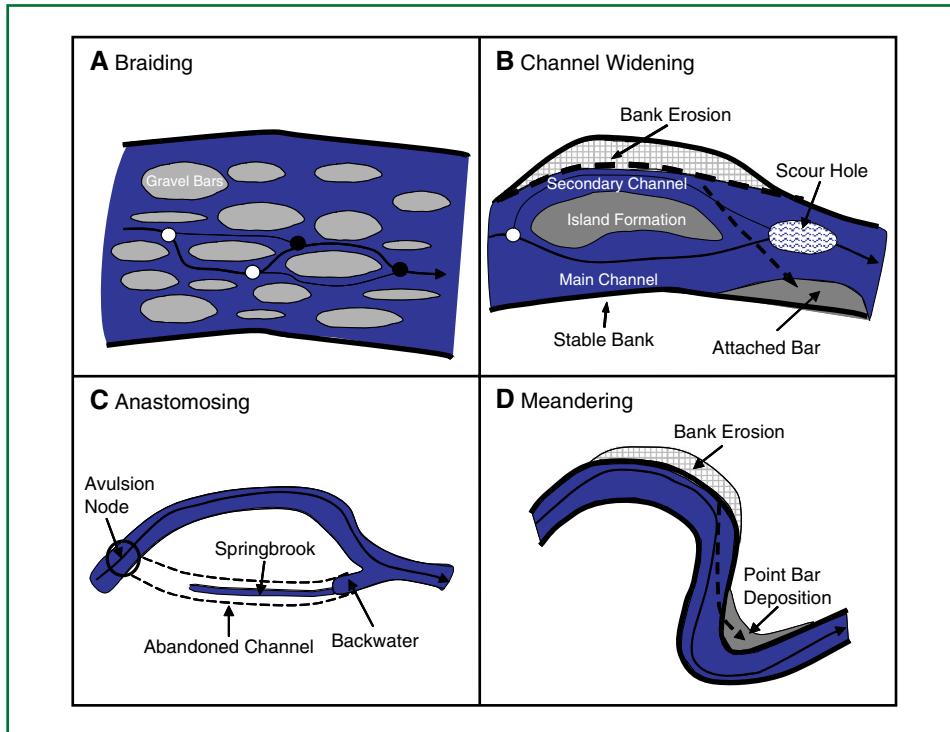
FIGURE 7.4 Fluvial geomorphologic pattern classified into four basic process categories: (A) Braiding, (B) Channel Widening, (C) Anastomosing, and (D) Meandering.

in successful recruitment of riparian vegetation through both seedlings and vegetative regeneration on newly deposited sediments (Hughes, 1997, Gurnell and Petts 2002, Gurnell *et al.* 2002). However, in braided rivers the likelihood of successful establishment of riparian vegetation over the long term is limited by frequent scouring floods. The Tagliamento River, a braided river located in northeastern Italy, has a turnover rate of 2.5 years for 30% of vegetated islands and a maximum age of vegetated islands of ~20 years. The degree of habitat change is controlled by flood magnitude, flood duration, and time between floods (Kollmann *et al.* 1999, van der Nat *et al.* 2003, Tockner *et al.* 2006). However, for the Tagliamento the presence of large wood and older vegetated islands was found to lead to an increase in habitat age by providing protected zones with more stable deposits within the harsh braided environment (Gurnell *et al.* 2001). What is interesting and important for the ecology of the Tagliamento, and most likely other rivers, is that in light of the high turnover rates of habitat the composition and diversity of aquatic habitats seems to remain relatively constant (Arscott *et al.* 2002, Tockner *et al.* 2006).

Channel Widening

As the supply of sediment relative to the capacity of the stream or river to transport that sediment decreases, identifiable channels emerge. These channels become more stable and high-frequency avulsion activity becomes a less dominant fluvial process. Channel-widening processes occur accompanied by the consolidation of bar deposition forming

either a midchannel island with a channel on either side or a shore attached bar. Lateral asymmetry in the flow field occurs resulting in erosion along the outside bend of a channel due to increased flow velocities along the inside of the bend (Figure 7.4B). Therefore, bank erosion and bar deposition begin to dominate and form a coupled feedback mechanism. Sediment eroded from a bank is deposited immediately downstream in the form of a laterally attached or midchannel bar (Figure 7.4B). The result of the bar deposition is the forcing of the channel toward the opposite bank, which in turn results in increased bank erosion along existing or newly eroding banks. This coupled process can force the river to widen but also begin to become more sinuous in plan form. Because of the active bank erosion and coupled deposition, this process becomes more reliant on the sediment supply from the immediate bank rather than supplies from the upper basin as in processes that lead to braiding. Often as the channel widens lateral bars grow until avulsion processes produce a more stable main channel with secondary channels and thus reducing the level of sinuosity. Several bars can coalesce as more sediment is supplied to the river resulting in increased avulsion activity on a larger spatial scale than found in braided systems.

The coupling of channel-widening processes with avulsion results in more complex fluvial environments from main and secondary channels with a wide range in water types from riffles, rapids, runs, and deep scour holes or pools formed by flow convergence. The formation and development of midchannel islands and shore-attached bars coupled with avulsion also produces other import water bodies like backwater channels, springbrooks, and parafluvial ponds. Floodplains dominated by a channel-widening process rather than braiding, while maintaining a frequently scoured parafluvial zone that limits vegetation, also produce conditions that stimulate the germination of seedlings on newly deposited bars. When channel avulsion or bar deposition forces the main scour activity of the river away from areas with regenerating riparian vegetation, then the condition exists where the seedlings can grow and mature (e.g., juvenile cottonwoods) on a gravel bar (Figure 7.2D). This leads to the situation where the gravel bar can become a trap for sediments and organic matter because the trees greatly decrease flow velocity that results in deposition of suspended sediments. This feedback mechanism between bar deposition, channel avulsion, and riparian succession will gradually build the gravel bars and eventually lead to a mature gallery forest within a passive orthofluvial zone (Stanford *et al.* 2005). Hence, it is the coupling of channel widening, bar deposition, and avulsion that results in a shift in the process trajectory from a parafluvial zone being dominated by annual scour processes toward a depositional process that turns the suite of parafluvial features (Figure 7.2) into a diverse array of habitats characteristic of the orthofluvial zone (Figure 7.3).

Anastomosing

Anastomosing is a term used to describe large-scale channel avulsions resulting in the creation of a new main channel or establish secondary channels (Schumm 1977). Either can bisect the orthofluvial zone through large-scale avulsions dominated by head-cutting processes that capture old channels more than create new ones (Figure 7.4C). In this category of fluvial behavior, braiding processes are essentially absent and channel widening is waning. Bank erosion continues, as well as bar deposition, but the ratio of sediment supply to transport capacity is smaller—hence both the main channel and secondary channels become deeper, more entrenched, and their position on the landscape becomes more stable. Sinuosity increases as a result of more persistent channels coupled with bank erosion and sufficient stream power to transport the available sediment supply.

Midchannel islands and lateral bars grow due to a shift in processes from annual scour to deposition as the riparian forests grow, mature, and continue to induce deposition from sediment-laden floodwaters and the accumulation of organic material. However, given the greater degree of stability and slow rate of habitat turnover, avulsion is still an important process in maintaining a complex array of springbrooks, flood channels, and backwater habitats (Figure 7.2). An important process here is that anastomosing rivers can bisect large islands and portions of the orthofluvial zone due to a scour process called head-cutting.

Head-cutting is a common scour process that can cause an orthofluvial flood channel or parafluvial secondary channel to redirect the main channel of a river. Head-cutting occurs when severe and rapid scouring of the bed develops in a localized zone within an existing secondary channel or flood channel and results in an upstream migration of down-cutting scour into the bed sediments. This down-cutting usually occurs at a point of sharp inflection between channel bed slopes and migrates upstream until it intersects the main channel capturing the river and diverting the flow to the head-cut channel. Head-cutting will cease when the overall gradient of the channel is diminished sufficiently to reduce the available stream power (Leopold *et al*. 1964; Chapter 4). The rapid flow of groundwater through the bed sediments of the head-cutting channel is an important aspect of the process—specifically the rate of head-cut migration that results when large differences in water surface elevations exist resulting in near fluidized conditions of the bed sediments. The large head pressures that cause the rapid flow of groundwater can transport fine sediments through the interstitial spaces, a process called piping or seepage (Leopold *et al*. 1964, Schorghofer *et al*. 2004). Large wood and wood debris dams are commonly associated with anastomosing channel avulsion caused by head-cutting. Often large wood (i.e., whole trees generally with attached rootwads) are deposited at the upstream ends of side or midchannel bars. The large wood functions as a flow resistance element and causes the water to slow and rise. This further increases the sediment deposition process, but it also locally raises the elevation of the water surface. This creates a localized high energy gradient with high head pressures affecting groundwater and head-cut processes in a nearby, hydraulically connected, secondary channel.

Meandering

Channel meandering is a process with similar elements to channel widening in which erosion occurs on the outside of the bank (Figure 7.4D). The primary difference between widening and meandering is that the pattern of erosion and deposition in meandering occurs in an orderly and repeated pattern of connected smooth curves. Channel avulsions do not occur, and the channel width remains relatively stable for long distances. Meander patterns typically occur in association with sand- and silt-sized material and result in long continuous connections of sinuous river bends. The degree of meandering or sinuosity can be quantitatively determined through the ratio of the stream length over the length of the valley (also see Chapter 4). Interestingly, meandering systems have a relatively high capacity to transport sediment (Bagnold 1960, Leopold and Wolman 1960). Meandering rivers can move across the floodplain in various manners. Typically the width of the channel remains constant, while the plan form of the meandering river progressively migrates. In most cases the channel forms a serpentine course of successive meander bends that migrate independently at different rates, forming tightly curved loops that cut through existing orthofluvial areas of a floodplain. Often a meander loop can turn back

on itself enough to cut off a meander loop and leave behind either a connected backwater or a completely disconnected meander arm called an oxbow lake.

The deposition of sediment on the inside of the meander bend forms a type of bar referred to as a point bar. As the channel meanders, a point bar can grow as a series of ridges and swales producing a scrolled point bar. A chronological sequence of riparian vegetation often develops across a point bar with the youngest seedlings near the river's edge and the older mature trees farther back along the migration axis of the meander bend. Channel meandering occurs on floodplains with relatively low slopes or energy gradients compared to the other three process categories.

B. The Role of Large Wood

Large wood (LW), often also referred to as large wood debris (LWD) plays an important role in river ecosystems (see Chapter 13), in part by influencing flow hydraulics, sedimentation patterns, and morphology (Gurnell *et al.* 2002 and 2005). A single piece of LW with an attached root wad or an aggregate of LW can act as an obstruction to flow creating a scour hole in the front of the obstruction and an accretion of bed sediments deposited in the lee of the obstruction (Figure 7.5A). Abbe and Montgomery
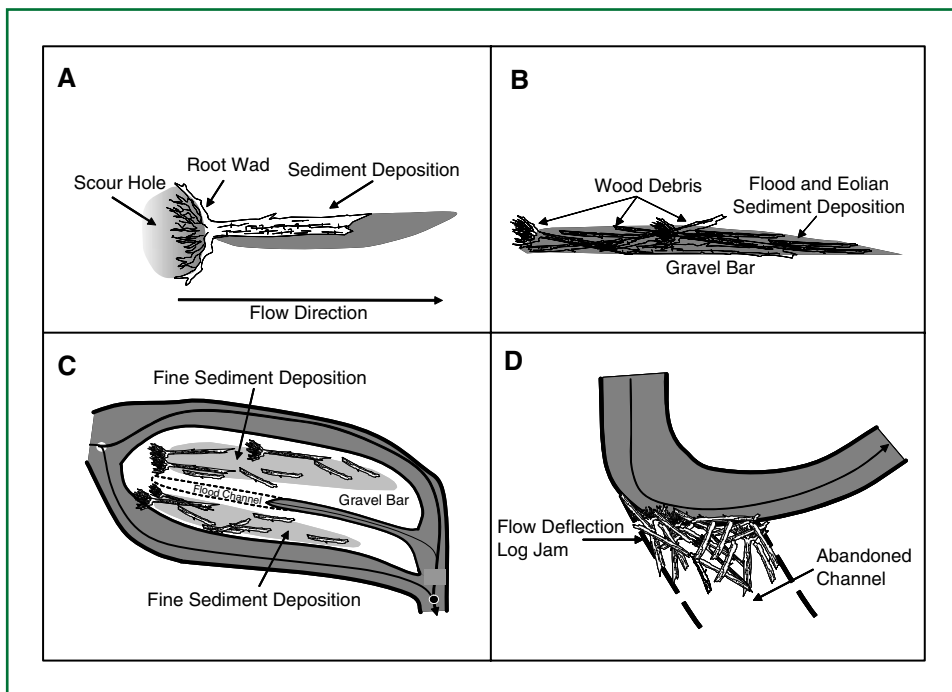
FIGURE 7.5 Illustrations of the interactions between large wood and the erosion and deposition of bed sediments. A. Typical position of scour hole (erosional process) in front of large wood with root wad and accretion (depositional process) of bed sediments deposited in the lee of the obstruction. B and C. Typical localized zones of sediment deposition producing "shadow zones" of sediment deposition where flow velocities are significantly reduced. D. Aggregate of large wood forming a log jam along the outside of a river bend. In this illustration the log jam is directing flow away from one channel and into another.

(1996) provide a methodology to relate the size of the scour hole to that of the LW and the flow hydraulics. The deposition of fine sediments provides favorable conditions for germination of seeds and sprouting of vegetative propagules that settle with the fine deposits in the lee of the LW. Likewise, LW deposits on bar tops can create localized zones of sediment deposition by producing "shadow zones" where flow velocities are significantly reduced, thereby inducing deposition of suspended sediments (Figure 7.5B and C). The presence of LW deposits not only causes scour holes to form and sediment deposition to occur, but these deposits can also deflect flows in a manner that maintains the position of secondary channels and flood channels (Figure 7.5C) or protection for older abandoned channels (Figure 7.5D).

Large wood can enter the floodplain through several mechanisms that include transport from upstream sections, erosion of lateral banks, and excavation of buried deposits by scouring floods. In this way LW acts as a feedback mechanism tied to the regeneration of new wood and production and maintenance of a supply of LW for the floodplain. Thus, LW dynamics are tied intimately to the dynamics of riparian vegetation regeneration and succession and the fluvial geomorphic processes of cut-and-fill alluviation and avulsion (Gurnell *et al.* 2005).

The categories of fluvial processes and the role of LW can be arranged along a continuum of floodplain channel typology and can be related to the relative rate of habitat turnover within the context of the primary drivers of geomorphic change: energy gradient (S_e), sediment caliper (D), transport capacity expressed as the ratio between sediment supply (Q_s) and stream power (Ω), or the product of shear stress τ and mean flow velocity v ($\Omega \equiv \tau^* v$) and the sediment source (Q_{Basin}/Q_{Bank}). These primary drivers of fluvial processes exist along a continuum of variation. We have grouped the continuum of resulting river typologies into three basic types (Figure 7.6) based on the dominance and overlap of the four classes of fluvial processes and the role of LW (Figures 7.4 and 7.5). Type I is dominated by channel braiding processes and has the highest relative rate of geomorphic change. The typology transitions from I to II as braiding processes decline and are replaced by channel-widening processes. In riverscapes with extensive riparian forests coupled with active cut-and-fill alluviating processes, the importance of large wood increases as a factor affecting scour and deflection of flow. LW also affects the processes controlling site regeneration of vegetation. As stream power and sediment supply decrease, so does the importance of large wood in geomorphic processes. The highest levels of geomorphic complexity and commensurate biological complexity and diversity appears in the Type II systems (Stanford *et al.* 2005).

III. SPECIFIC METHODS

A. Basic Method 1: Quantifying Fluvial Geomorphic Thresholds

Looking at a time-series of historical aerial photographs (Figure 7.7) one can identify changes in typology that can be used to infer past geomorphic processes and events. Channel avulsions can be ascertained and grouped into two types: parafluvial avulsions that produce and maintain secondary channels within the parafluvial and orthofluvial avulsion events that cut new or reoccupy old channels within the orthofluvial and completely abandon the previous parafluvial channel (Figure 7.7). We can see from the time-series of historical aerial photographs that a major changes occurred between each time interval represented from 1945 to 1999 and less change between 1999 and 2004. The parafluvial zone is greatly expanded over the ~20-year time span between 1945 and
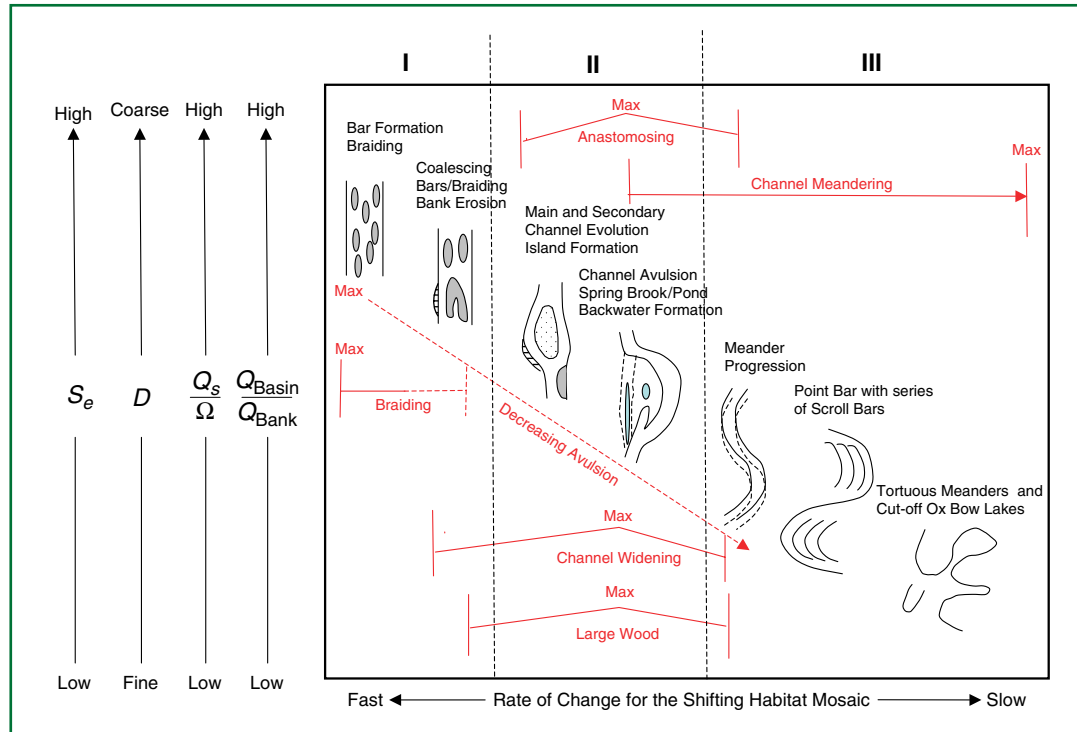
FIGURE 7.6 Categories of fluvial process and the role of LW arranged along a continuum of floodplain channel typology. These typologies are presented here within the context of the primary drivers of geomorphic change: energy gradient (S_e), sediment caliber (D), transport capacity expressed as the ratio between sediment supply (Q_s) and stream power ($\Omega \equiv \tau * v$, where τ is shear stress and v the mean flow velocity), and the sediment source (Q_{Basin}/Q_{Bank}).
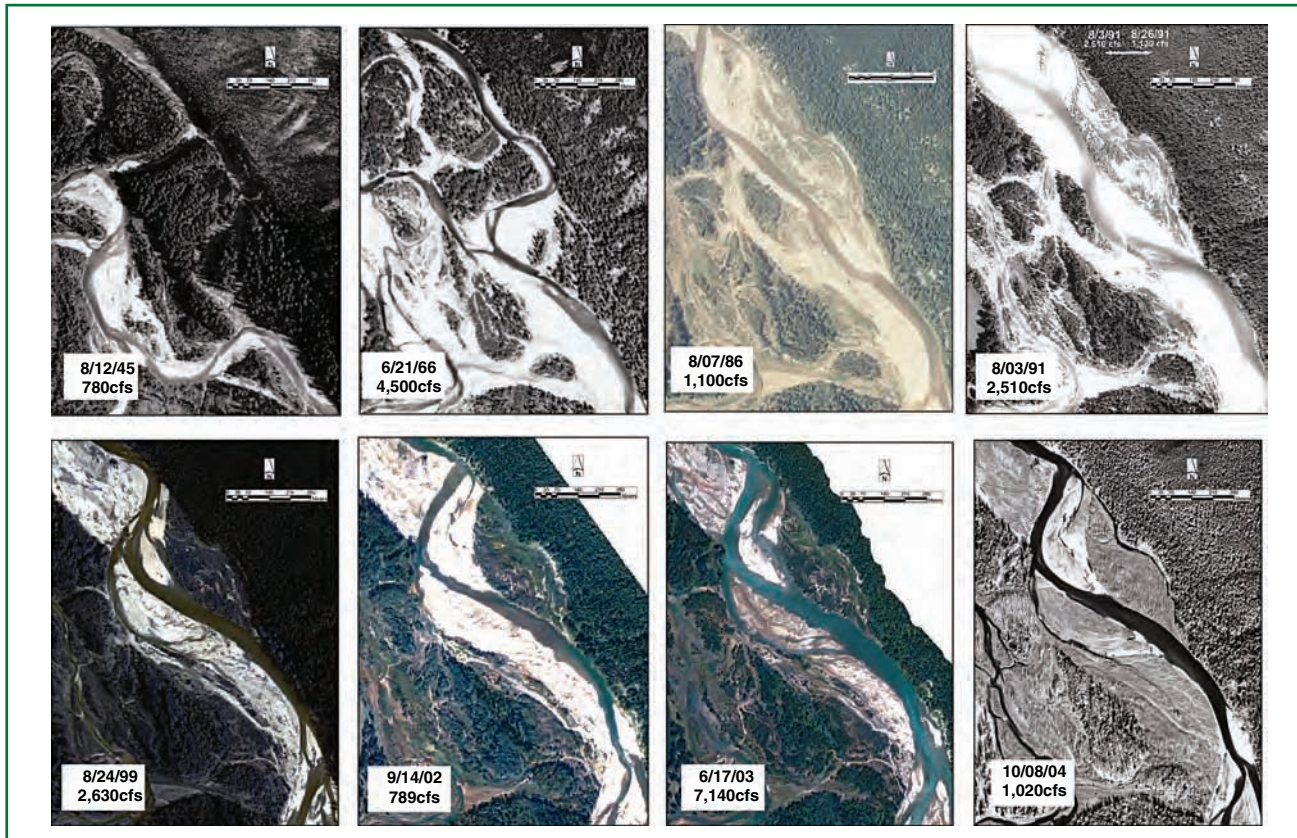
FIGURE 7.7 Time-series aerial photographs illustrating fluvial geomorphic change in the riverscape of a bar complex on the Nyack floodplain Middle Fork of the Flathead River, Montana. See Figure 7.1 for oblique view of the floodplain and location of aerial photos.

1966 coupled with major avulsion events. The following 20-year time frame is dominated more by parafluvial avulsion and widespread scour and bar deposition. This pattern of both parafluvial and orthofluvial avulsion, cut-and-fill alluviation and widespread scour tells us that this floodplain is dominated by channel widening processes, some anastomosing activity, and a fair amount of flow deflection and scour associated with the introduction of large wood. Channel-widening processes dominated both the five-year period between 1986 and 1991 and the nine-year period between 1991 and 1999. We can also see the deposition of gravel bars and loss of remnant orthofluvial islands due to bank erosion resulting in the realignment of the main channel but no major avulsion events. During the three-year period between 2001 and 2004 the most noticeable change is the rapid regeneration of riparian plants, mainly the establishment of cottonwoods (confirmed from ground surveys). With the exception of the 2003 photograph, all others are during base flow conditions. This is important to know when trying to make interpretations as to what types of geomorphic changes have occurred. For example, it would be incorrect to interpret that a channel avulsion had occurred between 2002 and 2003 based on the observation of flow visible as a secondary channel (Figure 7.7). This is because the discharge level in the 2003 photo was 7,140 cfs, nearly 10X the discharge during the 2002 photo.

Geomorphic change, for the most part, occurs when rivers flood and typically larger floods create more dramatic changes. Hence, to better interpret the changes noticed between photo years it is necessary to plot the peak discharge for each year spanning the time period of the photo series (Figure 7.8). This will tell you when the large floods
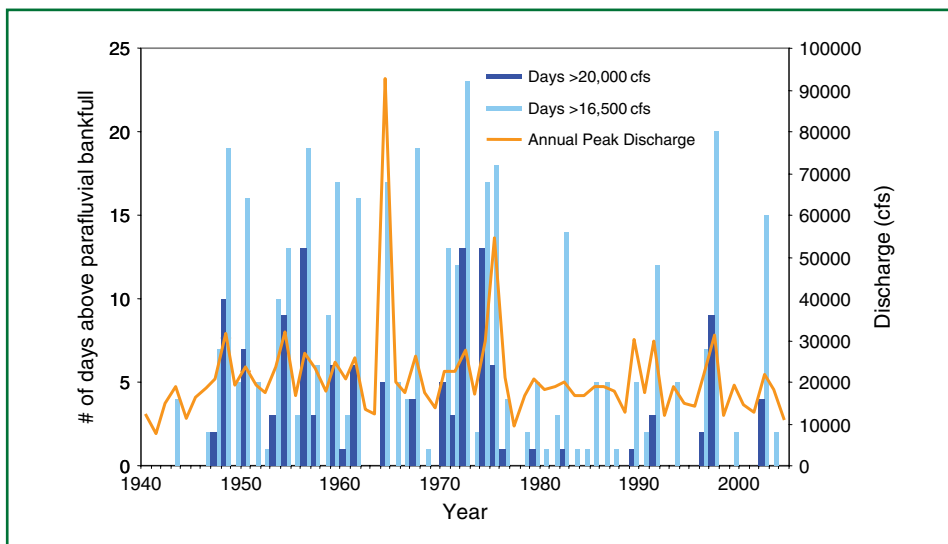

FIGURE 7.8 A plot showing the annual peak discharge (orange line) for the Middle Fork of the Flathead River from 1940 through 2004 taken from the USGS gaging station located in West Glacier, Montana, approximately 30 km below the floodplain. The period of record coincides with the aerial photographs in Figure 7.7. The number of days per year that discharge was at (light blue) or higher 10–20 cm higher (dark blue) than the parafluvial bankfull condition. For the Nyack floodplain, the parafluvial bankfull discharge starts around 16,500 cfs. Each 2000 cfs increase in discharge equates to about a 10 cm increase in stage height.

occurred relative to the major changes recorded in the photo series. For the floodplain illustrated in Figure 7.7, the 1964 flood was an extreme event and more than likely caused much of the change between 1945 and 1966. However, the peak discharge record alone cannot be used to explain all of the changes visible in the photo series. To get a better interpretation of the relationship between specific events and the discharge record, plot the number of days the discharge exceeds a geomorphic threshold where hydraulic work by the floodwaters can change the floodplain morphology. The bankfull discharge level is generally regarded as the critical discharge level for threshold entrainment of unconsolidated material where morphological changes begin to occur (Newson 1980, Lorang and Hauer 2003).

The 1.5-year return interval is considered a close approximation of the bankfull discharge magnitude for many river systems (Dunne and Leopold 1978). Other studies have found ranges between 1.5 and 25 years, with the wide range dependent on what flat surface of the floodplain was used to determine bankfull (Williams 1978, Nash 1994). The later studies used the low terrace of the active floodplain, which is by definition an abandoned geomorphic feature and would naturally lead to longer return intervals. Therefore, it is important to use the floodplain shelf of the active floodplain (Figure 7.9) to determine bankfull condition and associated discharge. (See Chapter 3 about calculating discharge (Q), hydraulic radius (R), slope (S), and flow resistance estimates.) The observation of flooding across parafluvial bar tops, connection of orthofluvial flood channels, and overtopping of floodplain shelves colonized with pole stand cottonwoods are all good indicators of a "bankfull" discharge, which for the floodplain in Figure 7.7 begins to occur around 16,500 cfs. This is really best described as a "parafluvial-full" condition and
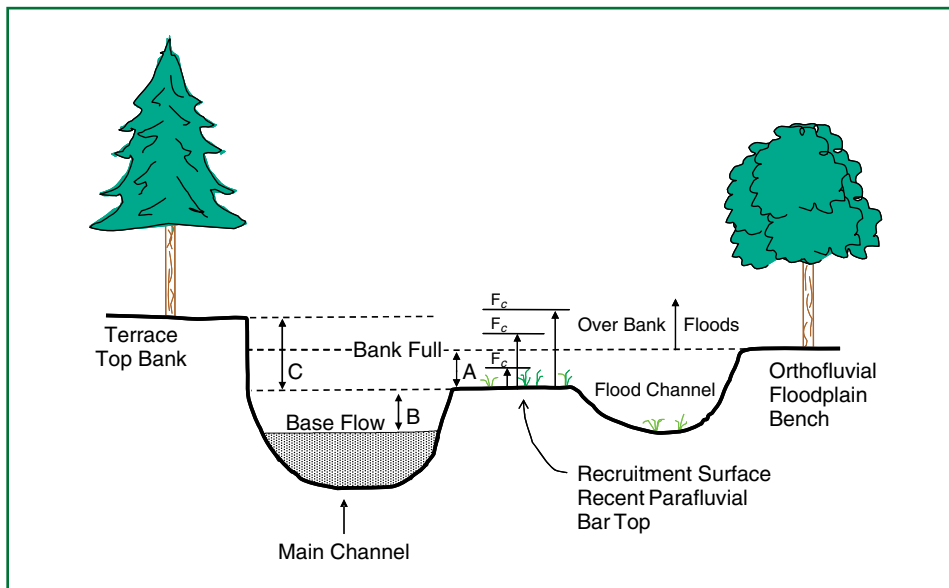

FIGURE 7.9 A sketch of an idealized lateral bank to bank cross section through a gravel bar showing differences in vertical elevations between various geomorphic features (bar top, top bank of active shelf, and top bank of the terrace). Also shown is elevation between the current water surface and the bar top and estimated flow competence water depths F_c estimated from the size of the rocks composing the bar top and estimates of S slope (e.g., water surface, across the bar, bar top, etc.) using Equation 7.3.

is the flow regime that often transports the greatest volume of sediment because much of the river has reached a level of flow competence (i.e., the level of flow where sediment transport begins; see Lorang and Hauer 2003 for greater detail). Therefore it is useful to also plot the number of days above "parafluvial-full" conditions. Orthofluvial avulsion events generally require higher peak flow events than a "parafluvial full" discharge, and some may require extreme flood events. However, the majority of geomorphic work is accomplished by longer-duration moderate events that achieve threshold-crossing flow competence levels (Bagnold 1977, Newsome 1980, Nash 1994, Lorang and Aggett 2005, Lorang *et al.* 2005). Where there is a well-developed parafluvial zone but not well-developed banks, one can estimate threshold entrainment discharge as occurring when most, if not all, of the gravel bars are inundated and where reconnection with orthofluvial flood channels begin.

Protocols

1. Obtain a complete set of aerial photographs and/or satellite images (resolution of at least 2.4 m such as Quickbird). Obtain scale and discharge level for each photo series.
2. Identify channel position or sites of primary flow, bank erosion and gravel bar deposition sites, and other geomorphic features identifiable on the photo images. Compare change in channel position due to avulsion processes versus widening or migration of the channel.
3. For each photo identify and quantify the number, length, and area of the following channel features where appropriate: (a) nodes of flow separation; (b) channel convergence; (c) length and area of main channel; (d) number, length, and area of secondary channels (be aware of the discharge level); (e) connected backwaters; (f) isolated ponds, lakes, and sloughs; and (g) springbrooks. Also compare the relative area of the parafluvial zone to the water surface for each photo.
4. Assemble existing discharge data for the nearest gauge. The USGS maintains stream gauging stations throughout the United States. Many other countries have similar services. To access these data in the U.S., go to website http://water.usgs.gov/ and in Canada http://scitech.pyr.ec.gc.ca/waterweb/. European countries have many different outlets for water discharge data. We recommend beginning with http://dataservice.eea.eu.int/atlas/. In New Zealand start with http://www.stats.govt.nz/analytical-reports/natural-resource-accounts/ water-natural-resource-accounts.htm and Australia http://www.nlwra.gov.au/.
5. Compare the time series photographs with the historic discharge data to identify likely periods of geomorphic change and the magnitude and duration of discharges occurring during photo series intervals.
6. Conduct the analyses of *Basic Method 1*, using a Geographic Information System. To do this accurately, aerial photos or satellite images must be georectified.

B. Advanced Method 1: Quantifying Fluvial Geomorphic Thresholds Using a Flow Competence Approach

After having gathered the available photo and discharge data and conducting the analyses of *Basic Method 1*, conduct a field survey to measure bar, channel, and bank topography of at least one gravel bar. Recall that typologies are presented here within the context of the primary drivers of geomorphic change: energy gradient (S_e), sediment caliber (D), transport capacity expressed as the ratio between sediment supply (Q_s) and stream

power (Ω), or the product of shear stress τ and mean flow velocity v ($\Omega \equiv \tau^*v$) and the sediment source (Q_{Basin}/Q_{Bank}) (Figure 7.6). The concept that is being tested in this method is what discharge level is required to begin to do significant geomorphic work (Figure 7.9). This analysis can then be tied to the duration component of the problem.

A flow competence approach is used to determine water depths and what related discharges are require to transport the sediments from the bar top and channel or to mobilize the gravel and how that discharge level compares with the local topography of the particular gravel bar. This will give a first order estimate of what the relative sediment balance is at the site, (e.g., is the site aggrading or filling in with sediment or is the channel incising or eroding further into the floodplain?). These simple approaches are important for assessing the impacts of dams, bank stabilization projects, roads, or logging and grazing practices that can disrupt a sediment supply and thereby impact the geomorphic condition of a floodplain (Lorang and Aggett 2005). A flow competence approach can be addressed, to first order, by starting with two separate equations;

$$\tau_{fl} = \rho g h S \tag{7.1}$$

$$\tau_{fc} = 0.045(\rho_s - \rho)g D_{mean}^{0.6} D_{max}^{0.4} \tag{7.2}$$

where equation 7.1 relates the potential force applied to the bed by the flowing water, called the shear stress, τ_{fl}, as a function of water depth h, channel slope S, density of water ρ, and gravity g. Equation 7.2 relates the necessary or flow-competent shear stress, τ_{fc} required to mobilize the gravel/cobbles composing the gravel bar or channel characterized by, ρ_s, the stone density and the intermediate diameter of the mean, D_{mean}, and maximum, D_{max}, size stones (Komar 1989). The threshold condition for sediment transport is defined when $\tau_{fl} = \tau_{fc}$. Making the proper substitute into the threshold condition and solving for h provides a single equation (7.3) that can be used to evaluate the flow depth required for flushing the sediments by allowing the river to do the work (Lorang and Aggett 2005).

$$h = 0.045\left(\frac{\rho_s - \rho}{\rho}\right) D_{mean}^{0.6} D_{max}^{0.4} S^{-1} \tag{7.3}$$

Once the topographic survey has been completed, cross-sectional plots can be made of the area and measures of differences between bar top and the orthofluvial bank top, and bar top and flow level the day of the survey, and bar top and the terrace top if one exists (Figure 7.9; see labels A, B and C). The next step is to determine the size of the material composing the top of the bar in order to solve equation 7.3 and compare calculated flow competence water depths with the local topography. Pick at least three locations across the top of the bar and place a 0.5×0.5 meter square on the substrate. Survey with the total station, the elevation of that location. You will be adding your calculated h values to determine the F_c elevations with local topography (Figure 7.9; see labels F_c). Next measure the intermediate axial diameter of the 10 largest stones within

the grid and make an estimate of what the mean intermediate diameter might be for all of the substrate within the grid. Use these data for the intermediate diameter of the mean D_{mean} and maximum D_{max} size stones in equation 7.3. Measure the mean slope over the length of the channel boardering the bar. Across the bar top use either or both of these for S in equation 7.3. Estimate stone density by measuring the mass of some small stones of similar lithology and the volume of water they displace in a graduated cylinder. Calculate a range of h values by using the ranges of values you have obtained for slope S and particle sizes (e.g., use the largest diameter for D_{max} and the mean of the 10 largest for D_{mean}).

Protocols

1. Using a surveyor's total station, survey the topography of your site, including measures of the water surface slope. The area covered should include at least one or two island or point bar complexes.
2. At the lab, download all the total station data into a format compatible for use in a GIS environment. Construct a contour plot of the topography over the georectified imagery obtained in *Advanced Method 1*.
3. Measure at least three grid locations on the bar top with the total station and measure the intermediate axial diameter of the 10 largest stones within the grid and make an estimate of the mean intermediate diameter for all of the substrate within the grid.
4. Using the total station in the GIS environment, construct topographic maps of the gravel bar and construct cross sections (e.g., similar to Figure 7.10) for each sediment grid location and or other locations of interest.

C. Advanced Method 2: Linking Fluvial Geomorphic Process with Field Attributes

After having gathered the available photo and discharge data and conducting the analyses of *Advanced Method 1*, conduct a GPS field survey to identify and map the distribution of biophysical attributes of the study site. Recall that typologies are presented here within the context of the primary drivers of geomorphic change: energy gradient (S_e), sediment caliber (D), transport capacity expressed as the ratio between sediment supply (Q_s) and stream power (Ω), or the product of shear stress τ and mean flow velocity v ($\Omega \equiv \tau^* v$) and the sediment source (Q_{Basin}/Q_{Bank}) (Figure 7.6). The concept that is being tested in this method is that changes in magnitude of any of these variables or their spatial distribution constitute the primary controls over the spatial distribution of aquatic habitats (e.g., see Chapter 2) and the distribution, abundance, and age structure of riparian plants (see Chapter 31); and the spatial distribution of groundwater–surface water interactions (see Chapters 6 and 33).

Protocols

1. Using a surveyor's total station and GPS, map the spatial distribution (patches) of sediment types, large wood, topography, and water elevation of ground water (i.e., potentio-metric surface). The area covered should include at least one or two island or point bar complexes.
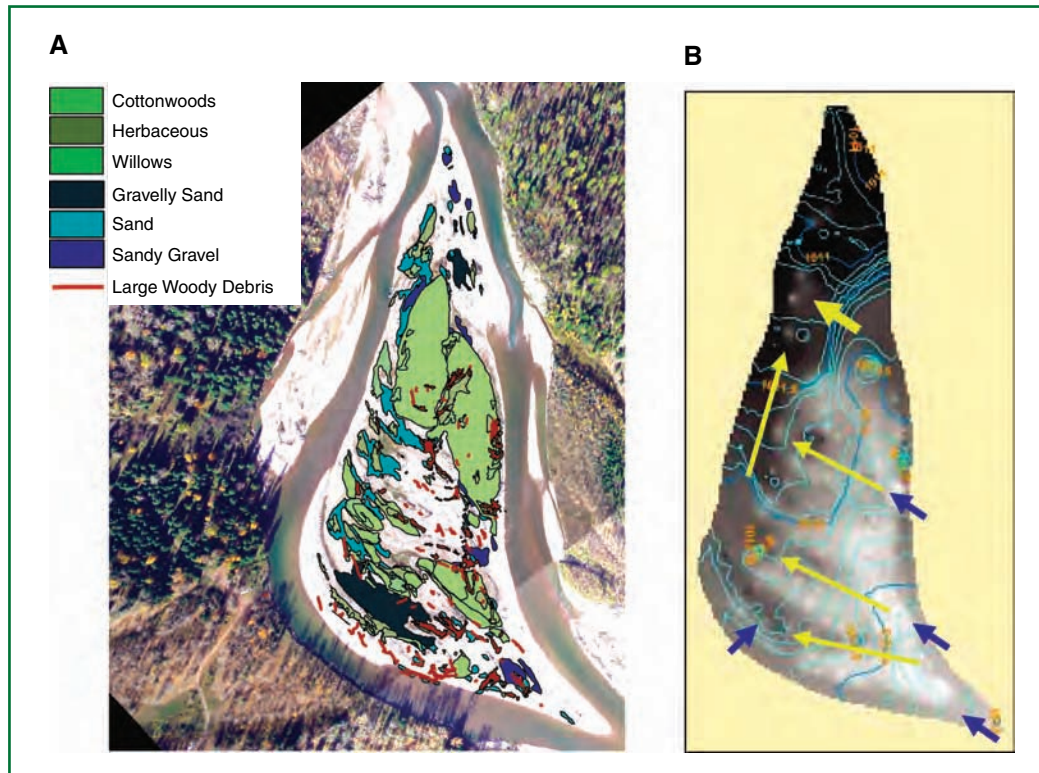
FIGURE 7.10 A. Distribution of trees, shrubs, and herbaceous plants, sediment patches and large wood as GIS data layers across the topographic surface of a channel bar. B. Contour plot of gravel bar subsurface water elevation and estimated direction of surface water infiltration and flow of hyporheic water through the bar.

2. At the lab, download all the total station and GPS data (points, lines, and polygons) and enter the data into a format compatible for use in a GIS environment. Construct a contour plot of the topography over the georectified imagery obtained in Advanced Method 1.
3. Using the total station and GPS data in the GIS environment, construct maps of the gravel bar that show the distribution of plant types (e.g., trees, shrubs, herbs) sediment patches (e.g., cobble, sand) and large wood. These should be constructed in the form of data layers draped across the topographic surface (Figure 7.10).
4. Over the same imagery construct a contour plot of sub-bar surface water elevation. Identify potentio-metric gradients (Figure 7.10).
5. Cross sections can also be made (e.g., plot of topography, water elevation coded to plant distribution) as well as many other data comparisons (e.g., cottonwood age versus depth to the water table) all driven by the specific questions of interest linking riparian ecology or GW-SW interactions or any other variables of interest to fluvial geomorphic process.

IV. QUESTIONS

1. What is the history of avulsion and cut-and-fill alluviation? How have image-identifiable floodplain attributes changed over time and space? What processes dominate your site and what is the relative rate of geomorphic change?
2. What types of discharge events are shaping your site, peak discharges and or longer duration parafluvial-full events? What role might large wood play?
3. Is your estimated threshold depth (F_c>A or C) or (F_c<A or C) or close to either A or C? What could that mean relative to the sediment budget for your site and how might human impacts, (e.g., dams, bank protection) be playing a role? How deep does the water have to be to scour the bar top? What type of errors might not be accounted for using such a simple algebraic equations (we refer the reader to Lorang and Hauer (2003) for an in-depth discussion of this question). Remember that simple equations like these are only meant to provide a quantitative tool; they are not right or wrong, so be careful in your interpretations. A simple plug and chug to get answer for *h* is just the starting point, not the final answer.

V. MATERIALS AND SUPPLIES

Aerial photo images
Satellite images
Access to discharge data for the site
Surveyor's total station
50 or 100 meter field measuring tape
0.5 × 0.5 pvc grid
Field GPS unit
Piezometers
Piezometer installing equipment (see Chapter 6)
GIS capable computing

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Solute Dynamics

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I. INTRODUCTION

Solutes are materials that are chemically dissolved in water. This includes cations (positively charged ions) such as calcium, magnesium, sodium, and potassium; anions (negatively charged ions) including chloride, sulfate, silicate, and bicarbonate; and organic molecules. In comparison to the common solutes, which are found in relatively large concentrations in many natural waters, more biologically important solutes such as phosphate and nitrate are normally at very low concentrations. Solutes enter streams from three natural sources. First, the atmosphere (i.e., rainwater) is often the major source of chloride, sodium, and sulfate. Second, other solutes come from soil and rock weathering, including calcium, phosphate, silica, and magnesium. Third, biological processes may be important. For example, while nitrate may enter from the atmosphere or from weathering, it may also be generated from nitrogen that was biologically fixed by cyanobacteria. Also, inorganic carbon (i.e., CO_2, bicarbonate, or carbonate) comes from the atmosphere and weathering, but it also comes from respiration by soil and stream organisms. Point sources (such as pipes) and non-point sources (e.g., agricultural runoff) are often major inputs of solutes to streams.

Solute dynamics refers to the spatial and temporal patterns of solute transport and transformation (Stream Solute Workshop 1990). These processes are tightly coupled to the physical movement of water in all ecosystems, but in streams this coupling is especially important. As materials cycle between biotic and abiotic components of stream ecosystems, they are continuously or periodically transported downstream. Thus the cycles are longitudinally drawn out to form spirals (Webster and Patten 1979, Newbold 1992). While the dynamics of many solutes are determined primarily by biogeochemical and hydrologic interactions occurring in the whole watershed (Webb and Walling 1992), important in-stream dynamics also occur (Peterson *et al.* 2001). Studies of solute dynamics in streams provide two types of information. First, they provide information on the rates of transport and transformation of the solutes themselves, which is important to the

FIGURE 8.1 Field setup for a solute release. This setup was used in a small agricultural stream in North Carolina. The 20-L carboy contained the mixed release solution, and the small table in the stream was used to stabilize the metering pump and battery. The solution was dripped into the center of the stream where the pink ribbon is tied to the hose. Photo by Rob Payn.

understanding of their availability and importance. Second, they can be used to quantify various hydrodynamic properties of a stream. In this chapter, we describe investigations of solute dynamics from both perspectives (Figure 8.1).

Solutes in streams can be classified in various ways (Stream Solute Workshop 1990). Nutrients are those solutes that are essential to the growth, maintenance, and reproduction of some organisms. Nutrients may be limiting to a given process if their concentration is too low to meet biological demand. Other substances such as heavy metals may be inhibitory or toxic to stream organisms. Stream solutes also can be classified according to their biological and chemical reactivity. If their concentration is changed by biotic or abiotic processes, they are referred to as *nonconservative* solutes. On the other hand, if their concentration is not changed by in-stream processes other than dilution, they are called *conservative* solutes. Conservative solutes include things that are not nutrients and do not react chemically with water or the stream substrate, such as lithium or bromide (e.g., Bencala *et al.* 1991). Also, some nonconservative solutes may be so abundant that biotic and abiotic exchanges do not measurably influence stream concentration, and the solute may appear to be conservative and may in fact be treated as a conservative solute. Chloride is an example of a biologically essential solute that exists in most streams in concentrations that far exceed biological need. Thus, chloride is often used as a conservative solute in stream studies (e.g., Triska *et al.* 1989).

A. Conservative Solute Dynamics

The dynamics of conservative solutes in streams are primarily driven by two processes; *advection* and *dispersion*. Advection is downstream movement with the water itself and occurring at the average water velocity. Dispersion can occur by molecular diffusion, but in streams is primarily caused by turbulence. The two processes are expressed in the partial differential equation:

$$\frac{\partial C}{\partial t} = -u\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2}$$

(8.1)

where C represents solute concentration; t, time; x, distance in the downstream direction; u, water velocity; and D, dispersion coefficient. However, this equation applies only to conservative solutes in uniform channels with constant discharge. Other terms can be added to this equation to include variable stream morphology, groundwater inputs, and transient storage. *Transient storage* refers to the temporary storage of solutes in water that is moving much more slowly than the main body of water (Bencala and Walters 1983), such as water in hyporheic flow paths, pools, and backwaters (Bencala *et al.* 1984, Harvey *et al.* 1996). Including these factors, the equation becomes a pair of equations:

$$\frac{\partial C}{\partial t} = -\frac{Q}{A}\frac{\partial C}{\partial x} + \frac{1}{A}\frac{\partial}{\partial x}\left[\frac{AD\partial C}{\partial x}\right] + \frac{Q_L}{A}(C_L - C) + \alpha(C_S - C)$$

and

(8.2)

$$\frac{\partial C_S}{\partial t} = -\alpha\frac{A}{A_S}(C_S - C)$$

where Q is discharge; A, the cross-sectional area of the stream; Q_L, the lateral inflow from groundwater; C_L, the solute concentration of the lateral inflow; α, a coefficient for exchange with the transient storage zones; A_S, the size (expressed as cross-sectional area) of transient storage zones, and C_S, the concentration of solute in the transient storage zone. Other metrics of transient storage can be derived from these parameters (Harvey and Wagner 2000, Runkle 2002). Because discharge (Q) and the cross-sectional area (A) are now changing with stream distance, they must be explicit in the equation.

Despite its apparent complexity, this model, like any model, is a simplification of what is actually occurring in streams. In reality there is probably a whole continuum of transient storage zones rather than a single transient storage compartment; however, this model has been shown to work well in many streams.

B. Dynamics of Nonconservative Solutes

Dynamics of nonconservative solutes are more complicated because of the production and consumption of solutes by in-stream processes. In small streams, the majority of these

processes occur on the stream bottom. They include abiotic processes, such as adsorption, desorption, precipitation, and dissolution. There are also many important biotic processes including heterotrophic (i.e., microbial) uptake, plant uptake, and mineralization. In general, abiotic and biotic processes that remove solutes from the water column are called immobilization. In streams the most important immobilization processes for biologically important solutes (i.e., nutrients) are adsorption (especially for phosphate), heterotrophic uptake, and attached algal uptake. Ignoring the complications we just added in Equation 8.2, the dynamics of a nonconservative solute can be expressed as:

$$\frac{\partial C}{\partial t} = -u\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} - \lambda_C C \qquad (8.3)$$

where C is the nonconservative solute concentration, λ_C is the overall dynamic uptake rate (units of inverse time), and other terms are as defined previously. Of course, nutrients that are immobilized may eventually be mineralized and returned to the water column. This can be most simply expressed by adding another term to Equation 8.3 and adding another equation for the immobilized nutrient:

$$\frac{\partial C}{\partial t} = -u\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} - \lambda_C C + \frac{1}{z}\lambda_B C_B$$

and

$$\frac{\partial C_B}{\partial t} = z\lambda_C C - \lambda_B C_B$$

(8.4)

where C_B is the immobilized (i.e., benthic) nutrient standing crop (mass per unit area), z is depth, and λ_B is the rate of mineralization.

These equations (or models) of solute dynamics can be much more complicated. This description was adapted from the more complete presentation generated by the Stream Solute Workshop (1990). The very simplest equation (Equation 8.1) can be solved analytically, but the other equations can be solved only by using computers and numerical solution techniques.

As a nutrient atom cycles between inorganic and organic forms, the spiraling length (S) is the distance it travels while completing this cycle (Newbold *et al.* 1981, Elwood *et al.* 1983). Over the length of a spiral, the nutrient changes from abiotic to biotic and back to abiotic form. Thus, the spiraling length has two components: (1) the distance traveled in dissolved inorganic form before it is removed from solution, called the uptake length (S_w), and (2) the distance traveled before being mineralized and returned to the water column, called the turnover length (S_B):

$$S = S_W + S_B \qquad (8.5)$$

Because much of the organic material in small streams resides on the benthic sediments (e.g., Fisher and Likens 1973) and movement of these particles is far slower than movement of dissolved constituents (Newbold *et al.* 1983, Minshall *et al.* 2000), the uptake length dominates total spiraling length in headwater streams (Newbold *et al.* 1983, Mulholland *et al.* 1985). Accordingly, the focus of this chapter is on the dynamics of dissolved inorganic nutrients as addressed by S_w and related measures.

Mathematically, uptake length can be related back to the previous equations as the inverse of the longitudinal uptake rate:

$$S_W = \frac{1}{k_W} \tag{8.6}$$

where the longitudinal uptake rate (k_W) is the dynamic uptake rate (λ_C) divided by water velocity:

$$k_W = \frac{\lambda_C}{u} \tag{8.7}$$

Because S_W represents a displacement distance, it is strongly influenced by stream discharge and velocity. To correct for the influence of stream size (i.e., discharge), S_W is often standardized when comparing solute dynamics across systems. This standardization converts S_W to a mass transfer coefficient (Steam Solute Workshop 1990), which describes a theoretical velocity at which a nutrient move towards the location of immobilization (e.g., the stream bed). More recently, the transfer coefficient has been referred to as the uptake velocity (v_f, Peterson *et al.* 2001, Valett *et al.* 2002). The uptake velocity corrects S_W for stream velocity and depth and is calculated as:

$$v_f = \frac{u\,z}{S_W} \tag{8.8}$$

Coupling v_f and ambient nutrient concentration (C) generates a measure of areal uptake (U):

$$U = v_f\,C \tag{8.9}$$

Areal uptake (mass per area per time) is a generic descriptor of nutrient cycling similar to measures used in other ecosystems. Uptake refers to the mass of an element taken up (or immobilized) by an area of streambed per unit of time. U reflects the magnitude of the flux of inorganic element from the water column to the biota.
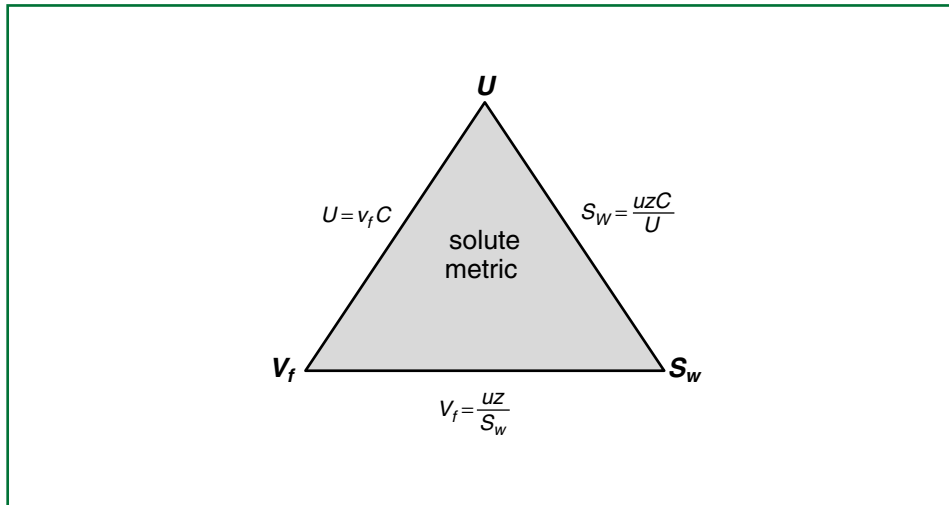
FIGURE 8.2 Metric triad for determining nutrient dynamics in stream ecosystems. Symbols are defined in the text.

Together, these measures (S_W, v_f, and U) form a metric triad of nutrient spiraling (Figure 8.2). Each metric has its utility in the study of nutrient cycling in streams. Areal uptake conveys critical information on biotic consumption but provides no information regarding the spatial aspects of nutrients. Uptake length is a reach or segment scale estimate of retention efficiency and provides explicit information regarding the spatial extent over which nutrient uptake occurs. Uptake velocity standardizes uptake length for discharge (depth and velocity) and provides a more appropriate variable for comparing solute dynamics across streams, although it is strongly influenced by nutrient concentration. Figure 8.2 also illustrates that v_f is a measure of uptake efficiency relative to nutrient availability (Davis and Minshall 1999), which can been seen by rewriting Equation 8.9 as:

$$v_f = \frac{U}{C} \tag{8.10}$$

The objective of the experiments described in this chapter is to examine the dynamics of both a conservative solute and a nonconservative solute (nutrient) in a stream or in a variety of streams. Because of the variability of equipment that might be available and the highly variable nature of stream chemistry, we have provided a number of procedural and experimental options. At a minimum, you should be able to determine discharge, velocity, the importance of transient storage, and an estimate of nutrient uptake. However, the estimate of nutrient uptake we describe here requires elevating the nutrient concentration, which may reduce nutrient uptake relative to supply (e.g., Mulholland *et al.* 2002). Measurement of nutrient uptake not influenced by raising the nutrient concentration above ambient levels requires the use of radioactive (e.g., Newbold *et al.* 1983) or stable (e.g., Peterson *et al.* 2001, Webster *et al.* 2003) isotopes. However, these techniques are time consuming, expensive, and sometimes not permitted. A new method was recently

described by Payn *et al.* (2005), which requires making multiple releases at varying nutrient concentrations. This new method involves significantly more effort, but it should be used if possible. The single nutrient addition method underestimates ambient nutrient uptake, but if used with care it can provide a useful method for comparing nutrient uptake across different sites or under variable experimental conditions.

II. GENERAL DESIGN

The general design of these experiments entails the release of a known concentration of solute at a constant rate into a stream for one to several hours and making measurements downstream to determine the longitudinal pattern of tracer concentration and the timing of the passage of the solute pulse (Figure 8.3).

A. Site Selection

Most solute studies have been done on first- to fourth-order streams that range in discharge from <1 up to 250 L/s. Streams this size allow wadeable access for physical measurements and sampling. Stream flows greater than this may require modification of the release apparatus and sampling design.

   Choice of a stream or section of stream will depend on the question posed (e.g., single reach or comparison of multiple reaches). Ideally, a stream or set of streams should be selected that provide a range of physical and biological conditions. A comparison of hydraulic properties between two reaches might include one simple reach (e.g., a straight channel with homogeneous substrate and low amount of wood) and one more complex reach (e.g., sinuous channel, heterogeneous substrate, high amount of wood). Try to avoid reaches with tributary input. Experimental reach length will vary with flow but minimally must be long enough for mixing and dispersion of released solute (a preliminary dye release may be in order). Typical lengths range from 50 m in very small streams to several hundreds of meters in larger systems.
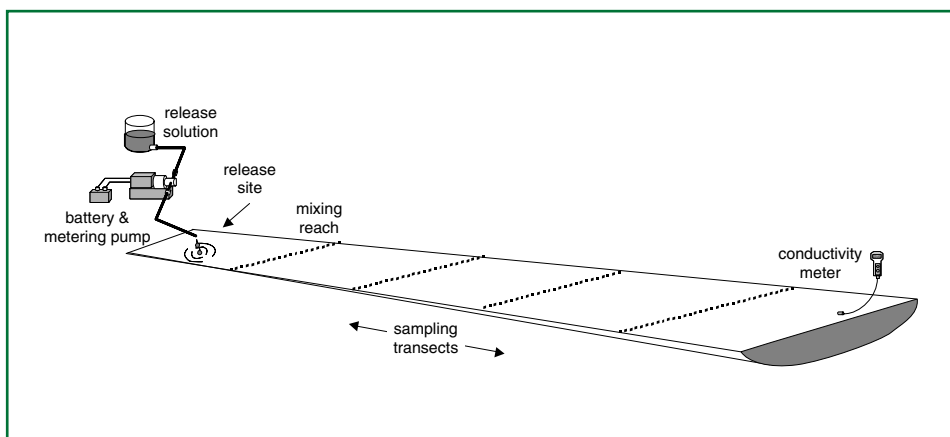

FIGURE 8.3 Diagram of the setup for measuring solute dynamics in a stream.

B. Choice of Solutes

Selection of a conservative solute is a function of local geology, ambient levels of solute in the stream, research budget, and analytical equipment available. It is essential to raise stream concentration of the solute sufficiently above background levels to be analytically detectable. Typical conservative solutes used are salts of chloride, sodium, lithium, potassium, and bromide. Of these, chloride is the most common. Chloride can easily be obtained as NaCl at a grocery store or feed store, but be sure it is noniodized. Most commercial NaCl contains a little cornstarch or other anticaking agents, which will cause a slightly cloudy solution but shouldn't be a problem. Chloride concentration can be measured in several ways. The most convenient way is with a conductivity meter, and conductivity itself can be used as a conservative measure (e.g., Mulholland *et al.* 1994). Conductivity is very sensitive to temperature, but most conductivity meters can be set to automatically adjust to temperature—that is, to measure specific conductance. Portable ion-specific probes are also available for chloride, bromide, sodium, and other ions. Bromide has the advantage of very low background concentrations and can be used in streams where background chloride concentrations are high; however, bromide ion-specific probes are usually less reliable than chloride probes and are influenced by changing chloride concentrations. The disadvantage of sodium is that it loses 5–10% by mass through sorption to stream bottom materials compared to almost no loss of chloride (Bencala 1985). If portable instruments are not available, samples can be collected in the field and analyzed in a laboratory by various spectrographic means.

Selection of a nutrient (nonconservative solute) for study will depend on your knowledge of the streams of interest. You will probably want to use the nutrient that is most limiting to autotrophic and heterotrophic processes, which might be determined with nutrient releasing substrates or experimental nutrient additions (see Chapters 10 and 32). Phosphate and inorganic forms of nitrogen (nitrate or ammonium) are obvious candidates. Your choice may depend on the availability of instrumentation for measuring concentrations of these nutrients. Be sure not to pick a nutrient that precipitates with the conservative solute. For example, calcium and phosphate cannot be used together because they form a highly insoluble salt.

C. Release Techniques

A simple, inexpensive, and nonelectrical release apparatus is the Mariotte bottle (Webster and Ehrman 1996), but battery-powered metering pumps (e.g., Fluid Metering, Inc., Syosset, NY, USA) are generally more reliable and are easily adjusted for variable field conditions.

D. Optional Approaches

Beyond the single reach release, solute dynamics can be compared spatially among the reaches of one to several streams, before and after manipulation, and over time at different flows. For each solute release, a computer model can be used to simulate the actual release data and calculate hydraulic parameters such as dispersion and transient storage zone retention. Nonconservative (nutrient) releases can also be run simultaneously with the conservative tracer. A computer simulation of the nonconservative solute dynamics also

may be run and compared with the conservative solute dynamics. Computer simulation methods are described later in this chapter.

E. Data Analysis

Essential physical measurements include discharge, average water depth, and average wetted-channel width for the stream reach over which the release is being conducted. Measurements of other physical parameters such as thalweg velocity, gradient, and "large wood area" or volume are optional. One can calculate hydraulic characteristics (discharge, velocity) from a graph of conservative solute concentration versus time from the results of the experiment; however, it is necessary to have a reasonable estimate of discharge prior to the experiment in order to calculate expected release concentrations. Uptake length and rate can be calculated from nutrient data fit to a negative exponential model. Further hydraulic properties of the reach (i.e., dispersion, transient storage zone area, and exchange rate) can be determined by curve fitting a computer simulation model to the conservative solute data. These techniques are described later in this chapter.

III. SPECIFIC METHODS

A. Basic Method 1: Dynamics of Conservative Solutes

In this example, we use chloride as the conservative solute and derive concentration from data obtained with a temperature-correcting specific conductance meter. For brevity, we call this conductivity.

Laboratory Preparation

1. Mix stock solution of sodium chloride in distilled water. A stock solution of 238 g NaCl/L (=144 g Cl/L) is two-thirds the saturation of NaCl in cold water and is fairly easily dissolved. Total volume needed depends on the number and duration of releases. Heating the mixture in a water bath aids in dissolution. Mix vigorously and repeatedly to be certain the salt is completely dissolved.
2. Prepare a series of chloride standards (1–20 mg/L) for calibrating the conductivity meter. Calibration involves constructing a standard curve that relates measured specific conductance to chloride concentration across the range of expected chloride concentrations.

Field Prerelease

1. Calculate stream flow and necessary release rate to raise stream concentration measurably above background. We've found that an increase of about 10 μS is generally sufficient if your conductivity meter reads to 0.1 μS. The necessary enrichment for solutes other than conductivity will depend on instrumentation and laboratory capabilities. Discharge can be estimated quickly from cross-sectional area and water velocity (see Chapter 3). The slug-injection method is another easy and

very accurate method of determining stream flow (Gordon *et al.* 2004). Once discharge is known, the release rate (Q_R) can be calculated as:

$$Q_R = \frac{Q * C_S}{C_I} \tag{8.11}$$

where Q is stream discharge; C_S, target stream concentration of added solute; and C_I, the concentration of solute in the release solution. Setting the pump to the calculated release rate will need to be done in the field, and the procedure is described in the next section.

2. Use a tape measure to delimit the extent of the experimental reach. Mark every 5 m (for a 100-m reach) within the reach with labeled flagging tape. At each 5-m transect, measure wetted channel width, depth across the stream (approximately 10 depth measurements at each cross section), and thalweg velocity (optional). Often, "effective depth" calculated from discharge, velocity, and width will be more useful than measured depth. Stream temperature and gradient (optional) should also be measured.

3. Calibrate the conductivity meter with the standards. The standards should be placed in the stream until they equilibrate with ambient stream temperature. Alternatively, conductivity (actually specific conductance) itself can be used as the conservative measure. To determine the conductivity of the release solution, make a 1:10,000 dilution (0.1 mL of release solution in 1 L of water) and measure conductivity.

Field Release

1. Make a series of background conductivity measurements in midstream at 10-m intervals (assuming a 100-m reach) along the reach. Work from downstream to upstream and avoid unnecessary disturbance of the study area. Then place the meter securely in a well-mixed area at the downstream site and assign a person to record conductivity during the release.

2. Place the release solution and pump at the upstream site. Check the pump rate with a graduated cylinder and stopwatch and adjust as necessary to the calculated release rate. Keep a bucket under the release hose to avoid any premature addition to the stream. During the release, periodically check and record the release rate, emptying collected solute in the graduated cylinder into the stream.

3. Be sure the release solution is stable and covered. Also, check the hoses to be sure they are secure and not laying in the stream.

4. Synchronize stopwatches and begin the release. The frequency of readings at the downstream site depends upon the rate at which the concentration changes in the stream. Record conductivity readings every 1–5 min (flow dependent) until pulse arrives and then every 15–30 s as chloride concentration increases rapidly.

5. At plateau, that is, when the conductivity is no longer changing (30 min to several hours after commencing release), working from downstream to upstream, again take measurements of conductivity at 10-m intervals. If you only have one conductivity meter, the break in the data at the downstream site won't be a

problem. After taking the upstream measurements, return the meter to the downstream site and shut off the release. Record the total time of release (i.e., the duration of the solute addition).

6. Continue recording downstream conductivity until stream levels return to near prerelease levels. We have frequently found that conductivity readings never return to background levels, either because of actual change in background or drift in the conductivity meter. To correct for either of these problems, it is useful to measure conductivity above the release site several times during the study.

Data Analysis

1. Summarize physical parameters: mean width and mean depth at each cross section and over the whole reach, mean velocity (optional), and gradient (optional).
2. Graph conservative solute concentrations versus time at the downstream end of the reach (Figures 8.4 and 8.5).
3. From this graph calculate discharge, Q, from plateau concentrations:

$$Q = \frac{(C_R - C_b) * Q_R}{C_p - C_b} \qquad (8.12)$$
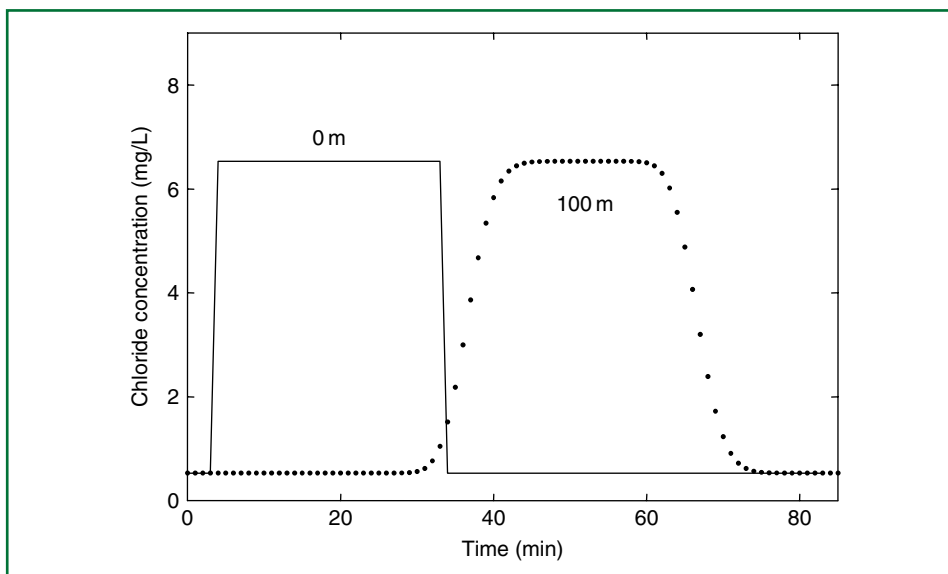
FIGURE 8.4 Chloride concentration versus time for a small stream with very little transient storage and no increase in flow over the reach.
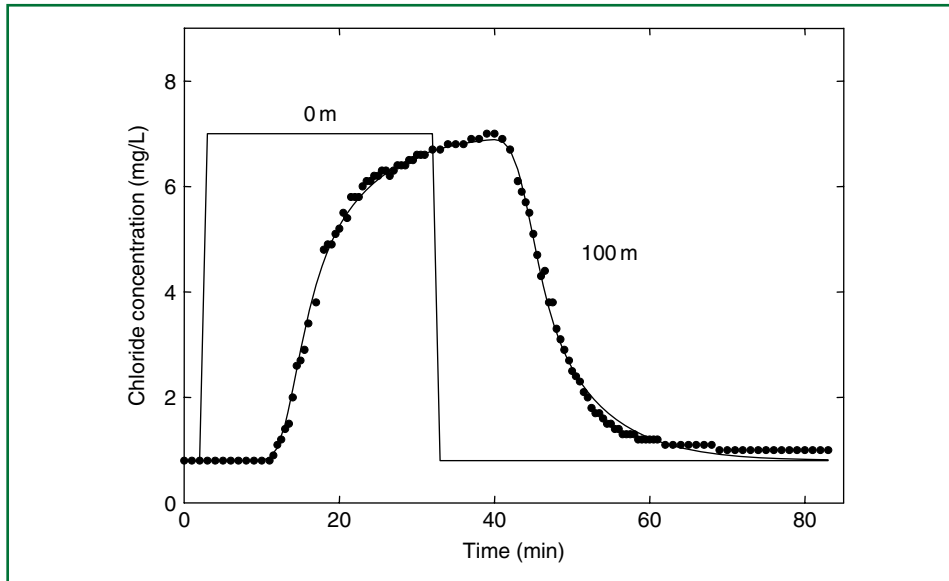
FIGURE 8.5 Chloride concentration versus time for a stream with considerable transient storage and no increase in flow over the reach. At 100 m the dots are actual data and the solid line is a computer simulation of these data using a transient storage model.

where Q_R is release rate; C_R, the chloride (or conductivity) concentration of the release solution; C_P, the plateau chloride concentration; and C_b, background (i.e., prerelease) chloride concentration. Compare this measurement of discharge with direct measurements. If conductivity is used, C_R can be calculated as 10,000 times the conductivity of a 1:10,000 dilution of the release solution.

4. A useful measure of hydraulic retention is the median travel time (MTT), which is the time required for 50% of the chloride to pass out of the stream reach (Runkel 2002). This can be determined by integration of the chloride curve (which can be done with many graphics or spreadsheet programs). Dividing the length of the reach by MTT gives the average solute velocity, which can be compared with direct measurements of thalweg velocity. For example, in Figure 8.4 the chloride release began at 12:04 and lasted 30 minutes. One hundred meters downstream, the solute pulse came by between 12:30 and 1:10. By integrating the curve, we determined that half of the added solute had passed by the 100-m point by 12:55. Since one-half of the 30-min release was completed by 12:19, MTT was 36 minutes (12:55-12:19). One hundred meters divided by 36 minutes is 4.6 cm/s.

5. Similarly, you can calculate discharge at points along the reach by using the plateau concentrations (Figure 8.6). Graph discharge versus distance to see if there is evidence of groundwater input. If there is evidence of flow increase at a specific point (or points), go back to the stream and see if you can identify landscape features associated with this subsurface input.

6. Comparison of your data to the curves in Figure 8.4 and 8.5 should give you some idea of the transient storage in your experimental reach. A reach with little or no transient storage will have a nearly rectangular graph (Figure 8.4). If transient
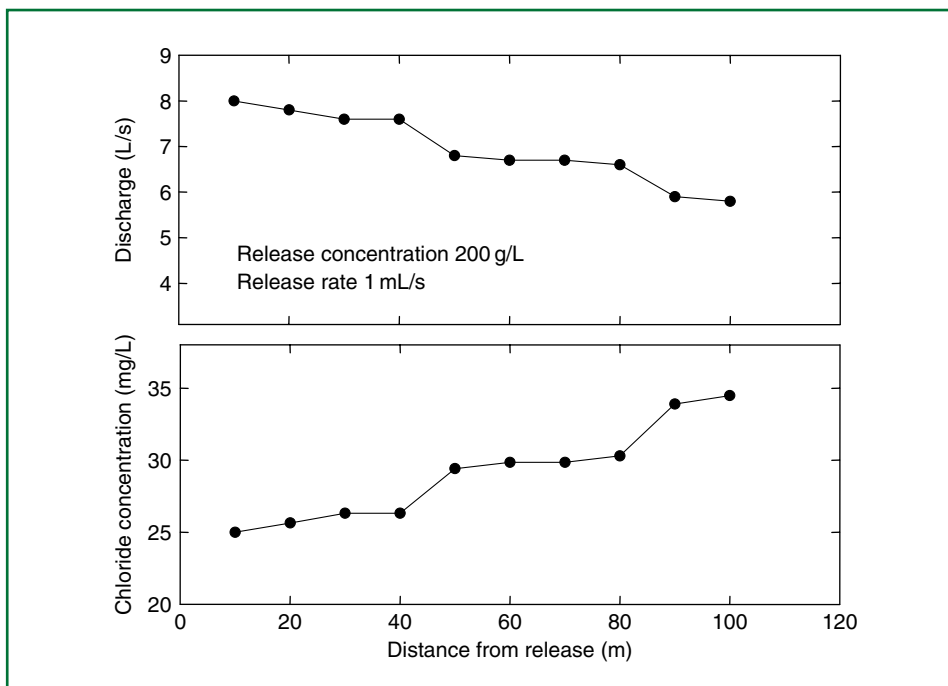
FIGURE 8.6 Plateau concentrations of chloride versus distance (top) and calculated discharge versus distance (bottom) for a stream with significant groundwater input over the reach.

storage is large, the uptake arm of the curve will have a rounded shoulder and the falling side of the graph will have a long tail (Figure 8.5).

B. Basic Method 2: Dynamics of Nonconservative Solutes

Simultaneously with the conservative solute, a nonconservative solute may be released to determine nutrient uptake. Determine the needed level of nutrient addition. Make a stock solution of nutrient, calculate the necessary release solution concentration based on the release rate previously determined for chloride, and add the appropriate amount of stock nutrient solution to the release solution. You will need to calculate your actual release chloride concentration as a result of dilution with the nutrient stock solution. As with the conductivity measurements, samples for nutrient concentration should be taken from the stream before the release and at the plateau of the release. Collect at least three replicate samples at each site. These samples can be taken in any type of clean container. If you are using glassware, acid washing will be necessary. We use disposable centrifuge tubes. The samples should be filtered either as they are collected or as soon as possible once the samples are taken to the lab. Methods of sample preservation vary depending on the nutrient you are using, and you should consult a manual such Standard Methods for the Examination of Water and Wastewater (Clesceri *et al.* 1998). In most cases it is best to keep samples on ice or refrigerated and analyze samples within 24 h of collection.
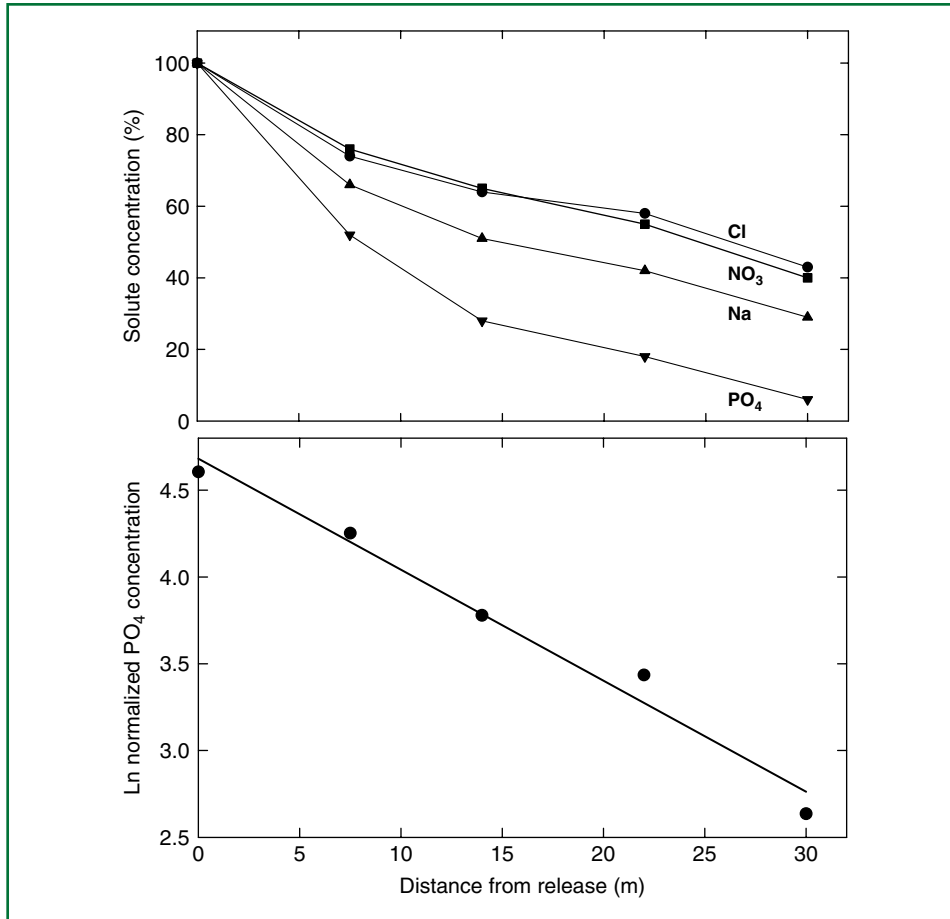
FIGURE 8.7 (Top) Plateau concentrations of solutes versus distance expressed as a percent of upstream concentrations. In this stream NO_3 was relatively abundant and acted like a conservative solute. PO_4 was rapidly taken up from the stream. (Bottom) Semi-log plot of normalized PO_4 concentration versus distance. The slope of this line is the PO_4 longitudinal uptake rate (k_L).

Graph normalized nutrient concentration versus distance and calculate the longitudinal uptake rate (k_W) and uptake length (S_W) (Figure 8.7). Nutrient concentrations of the samples collected at plateau must be corrected for background levels (C_b) in order to get the added nutrient level. Then calculate normalized added nutrient concentrations (C_N) by dividing the nutrient concentrations at a specific site (C_x) by the conservative solute (C_c, corrected for background) concentrations at the site:

$$C_N = \frac{(C_x - C_b)}{C_c} \tag{8.13}$$

By doing this you are essentially correcting for decline in nutrient concentration that may result from in-flow over the reach. For steady conditions (e.g., at plateau) the solution of Equation 8.3 is a negative exponential:

$$C_N = C_{N0}e^{-k_W x} \tag{8.14}$$

where C_{N0} is the added nutrient concentration at the release site, and x is distance downstream from the release site. Taking the logarithm of both sides of Equation 8.14 gives:

$$\ln(C_N) = \ln(C_{N0}) - k_W x \tag{8.15}$$

This is the equation for a straight line with intercept of $\ln(C_{N0})$ and a slope of k_W. So if you use your data to determine a regression of $\ln(C_N)$ versus x, the slope (k_W) will be an estimate of the longitudinal uptake rate, and uptake length (S_w) is the negative inverse of this (Stream Solute Workshop 1990). Uptake (U) and uptake velocity (v_f) can then be calculated using the metric triad (Figure 8.2).

C. Advanced Method 1: Computer Simulation

There are various computer models that can be used to simulate the results of your experiment (Figure 8.5). One example is a program called OTIS (One-dimensional Transport with Inflow and Storage), which is available on the web (http://webserver.cr.usgs.gov/otis). This simulation program was written by Robert L. Runkel, U.S. Geological Survey, Denver Federal Center, Denver, Colorado 80225, USA. It can be used to calculate transient storage parameters from the results of a conservative solute release experiment (Runkel 1998).

IV. QUESTIONS

1. What are causes of hydraulic retention in a stream? (That is, what causes temporary retention of conservative solutes?)
2. What stream features affect retention of solutes?
3. What factors determine the usefulness of various conservative and nonconservative solutes?
4. How does stream size affect hydraulic parameters?
5. What is the significance of wood in streams in terms of solute dynamics? How do you think the historical removal of wood from streams and rivers has affected solute dynamics?
6. Consider how various human modifications of streams and stream channels may affect solute dynamics. Think about such changes as nutrient enrichment from point sources or non-point runoff, channalization, dam construction or dam removal, and modification of riparian vegetation.

V. MATERIALS AND SUPPLIES

Laboratory Materials

> Conservative solute
> Nonconservative solute (nutrient)
> Carboy for stock solution of solutes
> Containers for standards
> Distilled water
> Graduated cylinders (100 mL and 1000 mL)

Lab Equipment

> Analytical instruments (for measuring solute concentrations)
> Computer (optional)
> Electronic balance (± 0.01 g)
> Filtering apparatus and filters

Field Materials

> Water-resistant paper or notebook, pencils
> Flagging tape
> Permanent marking pen
> Meter stick
> Tape measure (50–100 m)
> Stopwatches
> Thermometer
> Bucket
> Graduated cylinder (100 mL)
> Metering pump with tubing, battery
> Sample bottles
> Conductivity meter
> Chloride standards
> Velocity meter (optional)

VI. ACKNOWLEDGEMENTS

We thank the many current and former students who have worked with us conducting field studies of solute dynamics. Discussions with Dr. Steve Thomas were important in developing the metric triad. We are especially grateful to Father Terry Ehrman who made a major contribution to this chapter in the first edition.

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Phosphorus Limitation, Uptake, and Turnover in Benthic Stream Algae

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I. INTRODUCTION

Increased loading of nutrients into streams and lakes has become one of the major environmental problems facing society today. Nonpoint source pollution, associated with changing land use patterns and practices, has resulted in increased impairments to water bodies (e.g., Jordan *et al.* 1997, Carpenter *et al.* 1998, Allan 2004). These impairments include cultural eutrophication, harmful algal blooms, thermal pollution, increased sedimentation, and increased loadings of contaminants, such as pesticides, oil, and grease. The ability of an aquatic ecosystem to assimilate these stressors without exhibiting impairment depends largely on its biology, chemistry, geology, and geomorphology. In stream ecosystems, benthic algae and bacteria represent a potentially important biotic sink for pollutants, such as excess nutrients (Mulholland and Rosemond 1992, Bernhardt *et al.* 2003). Determining the rates at which nutrients are taken up and released can provide important information in assessing how large a nutrient load a stream, lake, or estuary can process before its integrity is negatively impacted (cf. Dodds 2003).

The nutrient that we focus on in this chapter is phosphorus. Inorganic phosphorus is commonly considered the element most likely to limit primary production in freshwater ecosystems (Schindler 1977, Hecky and Kilham 1988, Hudson *et al.* 2000). Although phosphorus concentrations in healthy plants are relatively low, usually ranging from

0.1 to 0.8% of dry mass (Raven *et al.* 1981), P is an essential element. Some of the more important functions played by phosphorus in plants include being a structural component of "high-energy" phosphate compounds (e.g., ADP and ATP), nucleic acids, several essential coenzymes, and phospholipids, as well as being involved in the phosphorylation of sugars.

Although a significant number of stream studies have indicated that phosphorus limits the growth of benthic algae (e.g., Stockner and Shortreed 1978, Elwood *et al.* 1981, Peterson *et al.* 1983, Bothwell 1989, Dodds *et al.* 1997), it is by no means the only limiting nutrient in lotic ecosystems. Nitrogen has been found to be the limiting nutrient in some streams (Grimm and Fisher 1986, Hill and Knight 1988, Lohman *et al.* 1991, Tank and Dodds 2003), whereas other lotic systems are colimited by nitrogen and phosphorus (Rosemond *et al.* 1993, Perrin and Richardson 1997, Francoeur 2001, Tank and Dodds 2003) or by micronutrients (Pringle *et al.* 1986).

In this chapter, three different aspects of phosphorus utilization by benthic algae will be covered: (1) assessment of P limitation, (2) measurement of P uptake rates, and (3) determination of the release rate of P (expressed as the turnover rate). We note two caveats regarding this chapter. First, we focus exclusively on inorganic phosphorus; it is likely that dissolved organic phosphorus (DOP) plays an important, albeit relatively undefined role, in the nutrient dynamics of freshwater algae (cf. Hwang *et al.* 1998, Pant *et al.* 2002). However, treatment of this topic is beyond the scope of this chapter. Second, although we use the term *benthic algae* throughout the chapter, it should be noted that the benthic algae attached to submerged substrata in streams usually exist as part of a complex assemblage variously referred to as *periphyton*, *aufwuchs*, or *biofilm*. This assemblage usually consists of algae, bacteria, fungi, and meiofauna (see Chapters 14, 15, 16, and 19) that exist within a mucilaginous, polysaccharide matrix (Lock *et al.* 1984).

A. Assessment of P Limitation

Nutrient limitation in algae can be assessed in several different ways, including elemental composition of biomass, nutrient enrichment bioassays, enzymatic activities, and physiological responses.

Elemental composition can suggest nutrient limitation because the proportions of carbon, nitrogen, and phosphorus, while confined to a relatively narrow range in algae (Hall *et al.* 2005), nonetheless vary in response to nutrient availability in the water. The ratios of carbon to nitrogen to phosphorus have profound ecological implications, as nutrient stoichiometry at the base of trophic food webs can influence trophic level interactions, population dynamics, taxonomic structure at the community level, and ecosystem level processes such as nutrient limitation and cycling (Hillebrand and Kahlert 2001, Sharfstein and Steinman 2001, Frost *et al.* 2002, Stelzer and Lamberti 2002). Kahlert (1998), in a review of the literature, found that the optimal (i.e., conditions without nutrient limitation or surplus) C:N:P ratio of freshwater benthic algae was 158:18:1 (molar), which deviates from both the Redfield ratio of 106:16:1 (Redfield 1958) derived from mixed phytoplankton populations and the ratio of 119:17:1 obtained for marine benthic microalgae (Hillebrand and Sommer 1999). It is likely that carbon-rich detritus (cf. Cross *et al.* 2003), the inclusion of macroalgae (cf. Hillebrand and Sommer 1999), and the carbon content of the mucilaginous biofilm matrix account, at least in part, for the higher C:P ratio of Kahlert's benthic algae compared to Redfield's planktonic algae. When carbon, nitrogen, or phosphorus become limiting in the environment, this can be reflected in a lower level of nutrient present in the algal cell. For example, if P

concentration becomes growth-limiting in a stream, tissue C:P and N:P ratios would be expected to increase because the algae make more efficient use of the P incorporated into cells. For freshwater planktonic algae, C:P values >129 and N:P values >22 (as opposed to Redfield ratios of 106 and 16, respectively) indicate at least moderate phosphorus deficiency in algae (Hecky *et al.* 1993). However, these ratios increase considerably for freshwater benthic algae, where phosphorus deficiency is suggested if C:P values exceed 369 and N:P values exceed 32 (Kahlert 1998).

Nutrient enrichment bioassays involve the addition of nutrients to a stream, either in the form of diffusing substrata (see Chapters 10 and 32), powdered fertilizers, or solute injections (see Chapter 8). The enrichment would continue for some designated period of time, and its effect would be evaluated by change in algal biomass (see Chapter 17) or primary productivity (see Chapter 28) compared to unenriched algae.

An enzymatic assay that has proven to be a reliable indicator of phosphorus limitation in algae is whole community phosphatase activity (PA). The phosphatase enzyme hydrolyzes phosphate ester bonds, thereby releasing orthophosphate (PO_4) from organic phosphorus compounds. PA is quantified by measuring the amount of hydrolysis produced after the phosphatase enzyme comes in contact with an added organic P substrate, thereby releasing PO_4. The most common type of phosphatase assayed in freshwater systems is alkaline phosphatase, which hydrolyzes phosphomonoesters. In contrast, phosphodiesterase hydrolyzes phosphodiesters, while adenosine triphosphatase hydrolyzes adenosine triphosphate (ATP). Increased PA results in more inorganic P becoming available to microorganisms in the environment. As inorganic phosphorus concentrations decline in aquatic ecosystems, PA generally increases (Healey 1973, Wetzel 1981, Currie *et al.* 1986, Espeland *et al.* 2002). Thus, PA has been used to infer P limitation for aquatic microflora (Healey and Hendzel 1979, Burkholder and Wetzel 1990, Newman *et al.* 2003). Based on their results from algal culture studies, Healey and Hendzel (1979) suggested that phosphatase levels above 0.003 mmol mg chlorophyll a^{-1} hr^{-1} indicate moderate P deficiency, and levels above 0.005 mmol mg chl a^{-1} hr^{-1} indicate severe P deficiency. It should be noted, however, that in systems with complex organic phosphorus substrates (e.g., dystrophic systems), PA can lead to biased conclusions if the assays do not include the appropriate phosphatases (cf. Pant *et al.* 2002).

The application of enzyme labeled fluorescence (ELF) has opened up new research avenues in the use of PA to detect phosphorus limitation. Although the details of this method are beyond the scope of this chapter, a brief review helps illustrate possible future directions of this field. Rengefors *et al.* (2001) used ELF to differentiate PA at the species-specific scale for phytoplankton. ELF results in a fluorescent product when it reacts with alkaline phosphatase at the site of enzymatic activity (i.e., cell surface). This method makes it possible to determine not only which species contain PA but also the relative quantity of PA. ELF also was applied to periphyton biofilms in a wetland to differentiate whole community phosphatase activity from that of individual bacterial cell phosphatase activity (Espeland *et al.* 2002). ELF labeling of both intact and homogenized periphyton mats may result in interesting insights regarding where phosphorus limitation is occurring within the biofilm matrix.

B. P Uptake Rates

The relationship between the nutrient concentration in the water and the rate at which nutrients are taken up by algae can be described by a hyperbolic function (Figure 9.1).
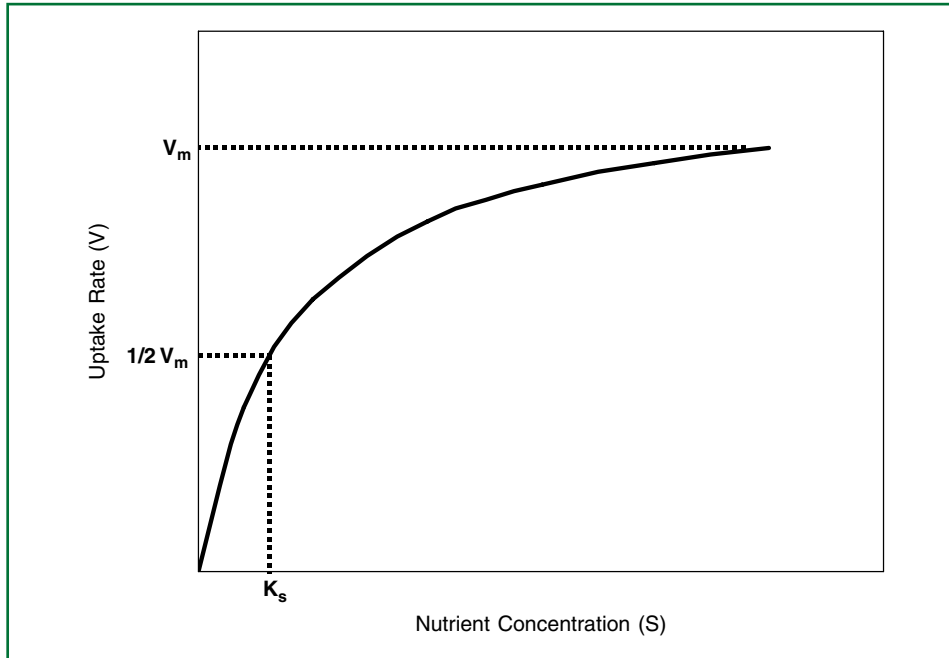
FIGURE 9.1 Relationship between nutrient concentration (S) and nutrient uptake rate (V). $V_{max} =$ the maximum nutrient uptake rate; $K_s =$ the half-saturation constant, or the nutrient concentration at which the uptake rate is one-half of the maximum uptake rate.

The Michaelis-Menten equation for enzyme kinetics is often used to describe this function:

$$V = V_m(S/(K_s + S)) \tag{9.1}$$

where $V =$ nutrient uptake rate, $V_m =$ maximum nutrient uptake rate, $S =$ concentration of the nutrient, and $K_s =$ the half-saturation constant (or nutrient concentration at which nutrient uptake is one-half the maximal uptake rate). From a biological perspective, there are two critical considerations in Figure 9.1. First, nutrient uptake rates become saturated as nutrient concentration increases. Empirical studies have shown that saturation of phosphorus uptake can occur at very low concentrations in both individual benthic diatom cells (<1μg/L; Bothwell 1989) and whole streams (<10μg/L; Mulholland *et al.* 1990). Thus, investigations examining P uptake in benthic algae must consider the possibility that saturation will influence uptake kinetics even at relatively low concentrations. Second, the constant K_s provides a useful index of a cell's affinity for a nutrient: a lower K_s suggests a greater affinity for the nutrient, which can confer a competitive advantage when the nutrient is present at low concentrations. Generally, taxa that have low K_s values have a competitive advantage at low nutrient concentrations. However, K_s values appear to be fixed, and do not appear to vary much under different environmental conditions. Rather, the physiological reason why nutrient-limited algae often increase

their short-term nutrient uptake rates when exposed to elevated nutrient concentrations is because of an increase in V_m and not a change in K_s (Darley 1982, Lohman and Priscu 1992). However, multiphasic P uptake systems have been documented in unicellular algae (Rivkin and Swift 1982, Jansson 1993), suggesting that K_s values are not static. From an energetic perspective, increasing V_m makes more sense, as a change in K_s requires the alteration of existing enzyme structures or the induction of an alternative enzyme, whereas increasing V_m requires only the activation or additional synthesis of an existing enzyme (Rivkin and Swift 1982). Of course, over the long-term, elevated nutrient levels may lead to an altered algal community structure, resulting in species with greater V_m or different K_s, thereby changing nutrient kinetics in the benthic algal community.

It is also important to distinguish between nutrient-limited *uptake* rates (above) and nutrient-limited *growth* rates. The relationship between nutrient concentration and algal growth can be modeled using either the Monod model or the Droop model. The Monod model relates algal growth to the external concentration of nutrients in the water, whereas the Droop model relates algal growth to internal (cellular) concentration of nutrients. For additional details on these models, see Droop (1974), Rhee (1978), Kilham and Hecky (1988), and Borchardt (1996).

C. P Turnover Rates

This portion of the chapter is designed to examine phosphorus turnover rates in benthic stream algae. Phosphorus turnover may provide an index of internal cycling in the algal community. Once an algal cell takes up phosphorus from the external medium, the P can be incorporated into structural elements, maintained in a labile pool, or excreted from the cell. Cells that are phosphorus-limited may be less likely to release the phosphorus they have taken up (back to the external medium) than cells that are phosphorus-replete (but see Cembella *et al*. 1984 and Borchardt *et al*. 1994). Thus, the phosphorus turnover rate in algae (i.e., loss of P from algal cell relative to total algal P) may be lower in P-limited cells than P-saturated cells, assuming both the P-limited and P-replete cells have similar metabolic activities and are exposed to similar grazing pressures (Steinman *et al*. 1995). One way to measure P turnover in algae is to label the cells with a P radioisotope (e.g., ^{32}P or ^{33}P) in the laboratory, place the algae back into the natural or a controlled environment, and then measure the amount of radioactive phosphorus present in the cells over time. This gives an apparent P turnover rate, as turnover is being estimated from the entire periphyton matrix and not from individual cells.

D. Overview of Chapter

This chapter examines phosphorus limitation and uptake in benthic algae collected from a relatively low- and a relatively high-phosphorus stream. In theory, the benthic algae growing in the low and high P streams should have adapted to the different ambient conditions, and exhibit different ecological attributes (Figure 9.2). Specifically, if the algae in the low P stream are P-limited, they should have low biomass and greater C:P ratios than algae collected from the high P stream, all else being equal. In addition, algae in the low P stream should have greater phosphatase activities, lower K_s values, lower total P uptake rates (i.e., computed as mass per unit time), and lower apparent P turnover rates (greater retention) than algae from the high P stream (Figure 9.2).
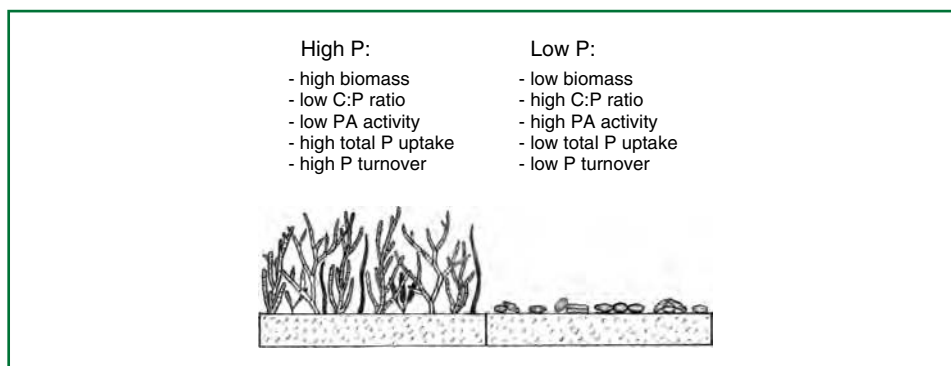
High P: Low P:
- high biomass   - low biomass
- low C:P ratio   - high C:P ratio
- low PA activity   - high PA activity
- high total P uptake   - low total P uptake
- high P turnover   - low P turnover

FIGURE 9.2 Phosphorus-related attributes for hypothetical attached algal communities exposed to high P (left side) and low P (right side) conditions.

II. GENERAL DESIGN

This chapter describes the methodology to measure phosphorus limitation, uptake, and turnover in benthic algae. Although valuable information will be gleaned from any of these methods in isolation, we recommend combining them when possible to gain a broader understanding of phosphorus-related processes in streams.

A. Site Selection

Both a relatively high-phosphorus (e.g. $>20\,\mu g$ PO_4-P/L, if available) and a relatively low-phosphorus (e.g. $<5\,\mu g$ PO_4-P/L, if available) stream are needed for this experiment. If differences in algal response are to be detected, it is critical that the algae be exposed to ecologically meaningful differences in nutrient concentration. We recommend using a relatively undisturbed stream (if available) for the "low phosphorus" system, where there are few obvious impacts (e.g., point source inputs, unnatural absence of riparian vegetation, livestock in streams). For the "high phosphorus" system, use streams receiving either agricultural runoff or point sources containing high P (e.g., sewage effluent), or clarifying tanks at sewage treatment facilities (Davis *et al.* 1990). If all streams in the region have low levels of P, then it may be possible to enrich a stream with P for a sustained period of time (e.g., $>4\,wk$) to create high P conditions (e.g., Steinman 1994, Cross *et al.* 2003). This can be done through the use of nutrient diffusing substrata (see Chapters 10 and 32) or solute additions (see Chapter 8). If all streams in the region have high levels of P, then we recommend that the two streams with the greatest difference in P concentrations be used. Regardless of which streams are selected, collect algae from sites in the two streams that are comparable in terms of other environmental conditions (e.g., irradiance level, current velocity, discharge, temperature, grazer density) to the greatest extent practicable.

B. Limitation: Phosphatase Activity (PA)

This method consists of two parts: an assay of phosphatase activity (PA) followed by measurement of chlorophyll *a* (see Chapter 17). The phosphatase activity is normalized per unit chlorophyll *a* to ensure that PA is not simply a function of how much (or how

little) active biomass is present. Phosphatase acts on a variety of organic phosphorus compounds. In this method, we add a commercially-available compound, *para*-nitrophenyl phosphate (*p*-NPP) to determine the PA present. When PO_4 is hydrolyzed from *p*-NPP, *p*-nitrophenol (*p*-NP) is formed, which can be measured spectrophotometrically.

C. Limitation: Chemical Composition (C:P ratio in algal tissue)

This method consists of three components: measurement of algal AFDM (and conversion to C), acid digestion of combusted matter to obtain dissolved phosphorus leached from ashed algal tissue (Solórzano and Sharp 1980), and then measurement of inorganic phosphorus in oxidized algal material according to standard methods. Ideally, the carbon concentration in algae would be measured with an elemental analyzer. However, this instrument is not always available. Consequently, in this method we present an alternative approach to estimate C, based on measurement of ash-free dry mass (AFDM; see Chapter 17), which is easy to perform but less accurate than elemental analysis. We assume C is 53% of AFDM, a reasonably accurate assumption for most algal communities (Wetzel 1983).

D. Net Uptake: Stable Phosphorus

This procedure involves the measurement of net loss of soluble reactive phosphorus (SRP) from the water in which the algae are growing. The method consists of three components: sample water during the incubation, measure SRP in water samples, and measure algal AFDM. Water is sampled at the start of the incubation, and thereafter at 30 and 60 min, and analyzed for SRP according to "Standard Methods" (APHA *et al.* 1995). Large changes in the biomass:water volume ratio resulting from water sampling during the incubation period should be avoided by minimizing sample volumes or number of samples. If the water volume in the incubation chambers is low, the 30 min samples can be omitted. We recommend that volumetric change be limited to <10% of initial volume during sampling. Ideally, chambers attached to pumps that could recirculate water during the incubation would be used (Figure 9.3), as water velocity will influence the uptake rate of phosphorus in benthic stream algae (Whitford and Schumacher 1964). However, if chambers and pumps are not available, the method can still be completed by using large (2 L) glass chambers and stir plates. An open petri dish is glued to the bottom of the chamber, into which is placed a stir bar. Then coarse-meshed screening (e.g., chicken wire) is placed over the petri dish, thereby creating a shelf onto which are placed the substrata with attached algae. The rotation speed of the stir bar is varied until it matches approximately the current velocity in the sampled streams.

The use of stable (i.e., nonradioactive) elements to measure net uptake rate is dependent on the initial nutrient concentration in the ambient water being high enough to measure the remaining nutrient at the end of the incubation period. For example, if P concentrations are low at the beginning of the incubation, they may be below the detection limit at the end. Another potential problem with this measurement is that if nutrient regeneration rates from algae are similar to nutrient uptake rates (i.e., the community is at steady state with respect to nutrient dynamics), then no net uptake will be measured, even if total uptake rates are appreciable. An alternative approach is to add nutrients to stream water. This elevates nutrient concentrations above ambient levels to ensure that concentrations at the end of the incubation will still be high enough to be measured, and temporarily increases nutrient uptake rates above rates of nutrient regeneration. However, this approach measures only nutrient uptake potential at the higher concentration
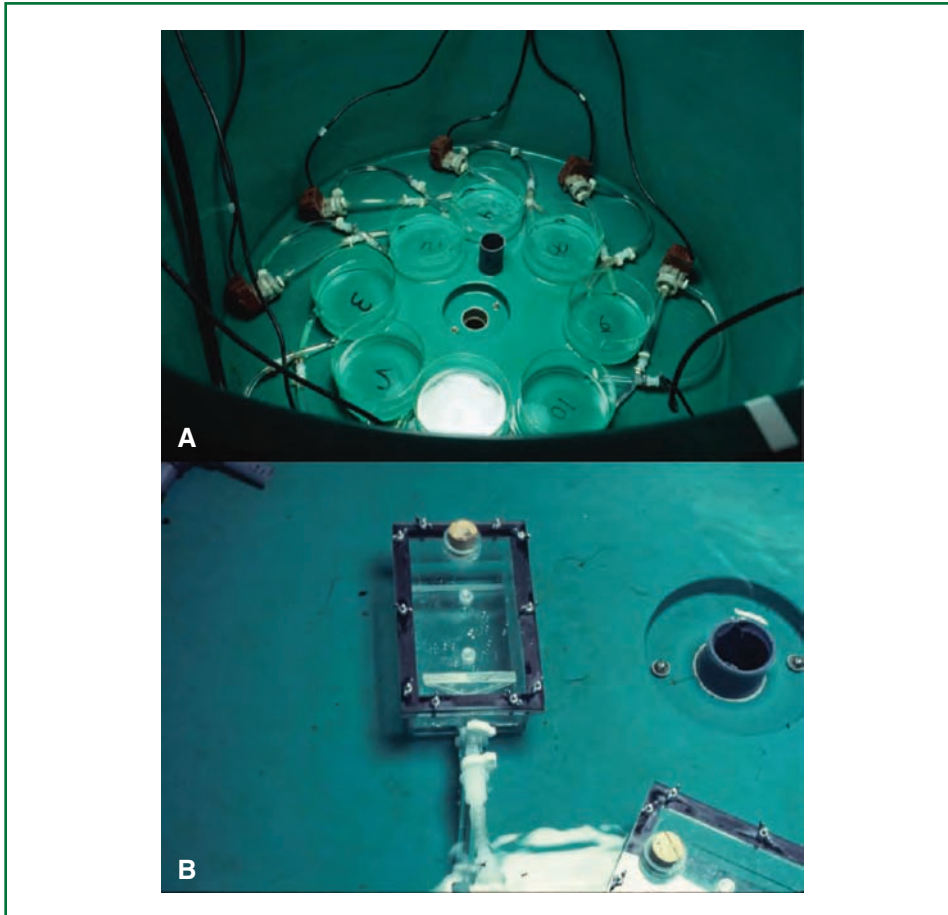
FIGURE 9.3 Examples of incubation chambers that have been used for P-uptake studies. (A) 2-L glass chambers fitted with adapters to accept tubing attached to submersible pumps. Pumps circulate water within the chambers. Placing adjustable clamps on tubing line can reduce flow rate, if so desired. (B) 1-L plexiglass chamber with detachable lid. Lid attaches to main body of chamber with wing nuts; gaskets provide a leakproof seal. Chambers are attached to submersible pumps. Note the large port (far end with cork) in lid, which allows an oxygen meter to be placed directly in the chamber to measure metabolism. There are also two small ports, which allow for injection of radioisotope into the chamber.

and may be an overestimate of ambient uptake rate depending on the degree of enrichment (see Mulholland *et al.* 1990, 2002). Alternatively, a nutrient addition approach that involves multiple levels of nutrient enrichment can be used to approximate total uptake rate at ambient nutrient concentration (Payn *et al.* 2005; see also Chapter 8). Here, we provide instructions for measuring net uptake rates at ambient nutrient concentrations (i.e., without enrichment).

E. Total (Gross) Uptake: Radioactive Phosphorus

This procedure involves measuring the loss of $^{33}PO_4$ added to the water in which the algae are growing. Two advantages of measuring nutrient uptake with radioisotopes,

as opposed to nutrient enrichment, are the ability to measure uptake at low ambient nutrient concentrations and the ability to measure total nutrient uptake rates.[1] Uptake rates will be calculated in this method from algae growing in high P and low P streams.

This method should use algae growing on small artificial substrata (e.g., unglazed ceramic cylinders [Steinman *et al.* 1991b] or unglazed tiles placed in the streams for a period long enough to acquire an algal community similar to natural substrata). The use of small artificial substrata allows AFDM to be measured directly on the substratum without it being physically removed, thereby minimizing contact with radioactive phosphorus in the algae. In addition, if the turnover option is to be completed (see following), the phosphorus in the algae on these substrata can be extracted with relative ease.

F. Turnover

This method involves measuring the rate at which radiolabeled P, incorporated into algal biomass, is lost from the algal assemblage over time.[2] Ideally, this procedure will be piggy-backed on the prior method of measuring phosphorus uptake rates using ^{33}P. The method consists of four parts: radiolabeling of algae, oxidation of labeled algae, extraction of P from ash, and measuring radioactivity in subsamples of extracted material.

After the ^{33}P uptake method is completed, substrata are either returned to the high and low P streams if possible, or placed into recirculating chambers or aquaria containing either high or low concentrations of phosphorus. Four substrata are sampled from each stream on four occasions over a 10-day period. The algae on the substrata are oxidized, and ^{33}P is extracted from the ash. A subsample of this extract is diluted, placed into scintillation cocktail, and assayed for radioactivity using liquid scintillation spectroscopy.

Phosphorus turnover for each stream is calculated as the first-order rate constant of the decline in ^{33}P activity over time (slope of relationship between $\ln[^{33}$P] in algae vs. time). A mean activity is calculated on each date from the four substrata collected and used in the regression with time. For the purposes of this method, we recommend normalizing ^{33}P content to unit area of substratum, as opposed to biomass, which assumes relatively similar biomass levels among substrata or that sufficient samples are collected on each date to take into account the natural variability of biomass in the system. If ^{33}P content is expressed per unit biomass, it becomes necessary to introduce a growth-correction factor to account for any net growth during the period of the experiment (because the amount of radioactivity per unit biomass in the sample will decline due to dilution by the accrual of new, nonlabeled biomass). Also, it is critical that if the extracted ^{33}P samples are counted on different days over the period of the turnover experiment, that they be corrected for radioactive decay from the start of the experiment; because of the short half life of ^{33}P (25 d), some of the decline in ^{33}P content in algae will be the result of

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[1] Extreme caution must be exercised when using radioisotopes. Users must consult with the radiation safety officer at their institution. We recommend the use of ^{33}P, instead of ^{32}P, because of its lower energy, although it is more expensive. Even with the relatively low maximum energy of ^{33}P (0.248 MeV), the small amount of radioactivity used (0.5 mCi/L), and the short half-life of the isotope (25 d), all handling of the isotope must be done with extreme care.

[2] Extreme caution needs to be exercised when using radioisotopes. See the prior cautionary note. In addition, because this exercise involves potentially placing radiolabeled algae back into the natural environment, we recommend that users consult with the radiation safety officer at their institution regarding restrictions or other potential concerns about this protocol. If this option is not viable, it is also possible to place the algae into chambers or aquaria filled with water of high and low phosphorus concentration, in order to evaluate the influence of P concentration on turnover.

radioactive decay. As a solution to this problem, all of the ^{33}P extract samples for the entire turnover study can be assayed on the scintillation counter at the same time at the end of the study, thereby eliminating the need to correct for decay.

III. SPECIFIC METHODS

A. Basic Method 1: Phosphatase Activity and Chlorophyll a

1. Preparation Protocol

1. At least one month, and preferably 3–6 months, prior to the experiment, place approximately one hundred $3 \, cm \times 3 \, cm$ unglazed ceramic tiles or ceramic cylinders (Steinman *et al.* 1991b; Du-Co Ceramics Co., Saxonburg, PA: http://www.ceramics.com/duco/) in selected pool and riffle habitats in the stream to be sampled. (The small size allows substrata at the time of sampling to be placed directly into an extraction jar without having to remove algae from the surface.) If unglazed tiles are used, and are purchased attached to each other in sheets (as opposed to individual tiles), place the entire sheet in the stream, which minimizes the likelihood of tiles being lost if high discharge occurs.
2. At least four tiles per stream should be analyzed. Alternatively, small rocks can be used but they must be small enough to fit in the incubation jars and be submersed in a small volume of water.
3. Label two Tupperware containers (ca. $30 \, cm \times 30 \, cm = 900 \, cm^2$) by stream name or type (high P; low P).
4. Label 18 wide-mouth glass incubation jars (30 mL or large enough to contain the substratum) by stream type and response metric (high P or low P stream; PA or chlorophyll *a*): one jar for each of the 4 tiles and one control per stream type for PA (10 total); 4 for each stream type for chlorophyll *a* extraction (8 total).
5. Prepare 150 mM *p*-NPP solution (add 2.78 g of *para*-nitrophenyl phosphate to 50 mL of double distilled water).

2. Field Collection Protocol

1. Collect tiles and filter water (filter about 500 mL of stream water into an acid-washed 1-L plastic bottle using a hand pump or 60 mL syringes and a Whatman GFF or Gelman Type A/E glass-fiber filter) from each stream.
2. Fill two acid-washed Tupperware containers (one labeled high P and the other labeled low P) with the appropriate stream water, and place ten tiles (two extra, in case of loss) from each stream inside the container. Attach the lids to completely water-filled containers (which minimizes tile movement), and place the containers in a cooler to be transported back to the laboratory.

3. Laboratory Protocol

1. Using an automatic pipette, transfer 20 mL of filtered stream water (use more water if needed to completely submerse substratum) to each of the 10 incubation jars labeled for PA (5 jars for each stream type). In the laboratory, separate the sheet of tiles into individual tiles (ignore any glue that may remain attached to individual

tiles following separation), and place one tile into each incubation jar. Leave one jar per stream without a tile (control).
2. Using an adjustable volume 1-mL pipette (set to 0.4 mL), transfer 0.4 mL of the *p*-NPP solution into the water in each of the incubation jars (or proportionately more if water volume is >20 mL), cap the jar, and gently mix. Incubate the jars at room temperature for 30 min, gently mixing the jars every 3–5 min.
3. After 30 min, filter the water in each jar by removing the water using a 25 mL plastic syringe and filtering it through a 0.45 μm pore size syringe-mounted filter (e.g., Syrfil-MF, Costar Corp., Cambridge, MA) and collecting 10-mL of filtrate in a labeled glass scintillation vial.
4. Remove the tile from each jar, rinse it by immersing it into unamended stream water, and place it in a small plastic jar or centrifuge tube containing a known volume of 90% acetone that is sufficient to cover the substratum for extraction of chlorophyll. Follow the procedures in Chapter 17 for chlorophyll analysis.
5. Add 0.05 mL of 1N NaOH to each vial containing the 10 mL of filtrate from each incubation jar to bring the pH up to ~10 (for maximum color development of nitrophenol). Measure the absorbance of each filtrate at 410 nm against distilled water using a dual-beam spectrophotometer and a 1 cm pathlength cuvette.

4. Data Analysis

1. Phosphatase activity (PA) is calculated from the absorbance of the NPP solution as follows:

$$PA = (Abs_{sample} - Abs_{blank}) \times 58 \times Volume_{(inc.)} \tag{9.2}$$

where Abs_{sample} = absorbance reading of sample at 410 nm, Abs_{blank} = absorbance reading of control at 410 nm (filtered stream water only, to correct for natural phosphatase in water), $Volume_{(inc.)}$ = volume of stream water in which each algal sample is incubated (in L); [If 20 mL is used (as described in this method), this value will be 0.02]. Use Table 9.1 for data entry and calculations.
2. The value 58 in equation 9.2 is the specific absorbance (at pH >10) of nitrophenol, which is the hydrolysis product of *p*-NPP. The phosphatase activity thus calculated is then divided by the amount of chlorophyll *a* determined for each sample, to obtain chlorophyll-normalized PA (with units of mmols mg chlorophyll $a^{-1}1/2\,hr^{-1}$). If phosphatase levels are very low, the incubation period can be extended to one hour, and the values are reported per hr. Alternatively, PA could be normalized by tile surface area to obtain area-specific PA (units of mmols $cm^{-2}1/2\,hr^{-1}$).

B. Basic Method 2: Chemical Composition

1. Preparation Protocol

1. Label two acid-washed Tupperware containers (30 cm × 30 cm) per stream by stream name or type (high P; low P), resulting in a total of four containers.

TABLE 9.1	Sample Data Sheet for Determination of Phosphatase.

	Stream					
Sample	Absorbance (410 nm)	Net Abs. (sample blank)	Volume (liters)	Phosphatase Activity (mmol/hr)	Chlorophyll *a* (mg)	Chl-Specific Phosphatase (mmol mg^{-1} h^{-1})
Blank						
1						
2						
.						
.						
n						

2. If tiles are to be used for this experiment (in lieu of rocks), place approximately one hundred $3 \, cm \times 3 \, cm$ unglazed ceramic tiles or ceramic cylinders (Steinman *et al.* 1991b) in selected high-P and low-P streams to be sampled. Tiles should be placed in streams at least one month, and preferably 3–6 months, prior to the experiment. If tiles are used, they should be pre-ashed to remove attached glue, which otherwise would be included in the AFDM measurement.

2. Field Collection Protocol

1. Collect rocks or ceramic tiles from each stream.
2. Fill the two Tupperware containers (one labeled high P and the other labeled low P) with stream water and place three small rocks from each stream inside the container. Attach the lids to completely water-filled containers (which minimizes rock movement) and place the containers in a cooler to be transported back to the laboratory.

3. Laboratory Protocol

1. Follow the general procedures outlined in Chapter 17 for determination of AFDM, including the following modifications. After the algae are removed from each rock, add the slurry (make sure the volume is less than 10 mL) to the bottom of an acid-washed 10-mL tared, glass beaker. Cover the top of the beaker with aluminum foil and lightly etch the sample number onto the foil with a pointed object (do not write it on the beaker because it will burn off during combustion, potentially contributing to dry mass and leaving one unable to track individual samples). Dry the beaker to constant weight at 105°C (ca. 24–48 hr). Remove the beakers from the drying oven and transfer them to desiccators until weighing.
2. After the beakers have been weighed, place them in a muffle furnace at 500°C for at least 4 hr (make certain the oven is at 500°C before timing), remove, and allow beakers to cool to room temperature in a desiccator, and reweigh.

3. Using a 5 mL pipette, add 5 mL of 2N HCl to the beaker, label the beaker with the sample number, and replace the aluminum foil with parafilm over the beaker to prevent evaporation. Acid extraction of ashed material should last at least 24 hr. Place beakers in the laboratory hood during the extraction period.
4. After extraction, transfer contents of each beaker to a 500 mL volumetric flask. Rinse the beaker with distilled water and pour rinse water into the volumetric flask as well. Bring the total volume in the volumetric flask to 500 mL by adding double distilled water (this will result in a leachate of 0.02N HCl).
5. Pour each sample into separate plastic bottles, label accordingly, and analyze using standard methods for analysis of phosphorus in water (APHA *et al.* 1995).[3]

4. Data Analysis

1. Calculate the amount of carbon in the sample by multiplying the AFDM by 0.53. (Carbon content is estimated by assuming that 53% of AFDM is comprised of carbon [Wetzel 1983]. Although this value may vary slightly among algal groups and environmental conditions, the variance is low [±5%] compared to other cellular constituents). Use Table 9.2 for data entry and calculations.
2. Calculate the concentration of phosphorus in each sample by comparing its absorbance against a standard curve developed from the standards analyzed. The total amount of P (in mg) is then calculated by multiplying the P concentration by 0.5 (because the total volume of diluted leachate is 0.5 L).

TABLE 9.2 **Sample Data Sheet for Determination of Chemical Composition (Italicized Letters in Formulae Refer to Column).**

Sample	Stream					
	A	*B*	*C*	*D*	*E*	*F*
	Beaker + Dried Material on Filter	Beaker + Ashed Material on Filter	AFDM = *A − B*	Carbon (mg) (AFDM × 0.53)	Phosphorus (mg) from Digestion and SRP Analysis	Molar C:P [(**D/E**) × 2.58]
1						
2						
3						
.						
.						
.						
n						

---

[3] Standards for P analysis must be made in 0.02 N HCl to be comparable to that of samples.

3. The C:P ratio is calculated by dividing the total C by the total P in each sample
    (converted to the same mass units) and then multiplying by 2.58 (to convert to a
    molar basis). Compare the ratio to the Redfield ratio (106:1) and analyze the
    differences between the high-P and low-P streams.

C. Advanced Method 1: Net Nutrient Uptake—Stable Phosphorus

1. Preparation Protocol

1. At least one month, and preferably 3–6 months, prior to the experiment, place
    approximately one hundred $3\,cm \times 3\,cm$ unglazed ceramic tiles or ceramic cylinders
    (Steinman *et al.* 1991b) in selected high-P and low-P streams to be sampled. If tiles
    are used, they should be pre-ashed to remove attached glue, which otherwise would
    be included in the AFDM measurement.
2. Label two acid-washed Tupperware containers ($30\,cm \times 30\,cm$) by stream name or
    type (high-P; low-P).
3. Label 6 acid-washed 50 mL collection bottles according to treatment (high-P vs.
    low-P) and time (initial, 30, and 60 min).

2. Field Collection Protocol

1. Collect tiles and filter water (filter 1 L of stream water into an acid-washed 1-L
    plastic bottle using a hand pump and a Whatman GFF or Gelman Type A/E
    glass-fiber filter) from each stream.
2. Place ten tiles into a labeled Tupperware container (high-P or low-P) per team,
    which is filled with stream water. Attach the lids to completely water-filled
    containers (which minimizes tile movement) and place the containers in a cooler to
    be transported back to the laboratory.

3. Laboratory Protocol

1. Transfer the 1 L of filtered stream water and tiles into each stirred or recirculated
    incubation chamber (the number of tiles placed in the chamber is dependent on the
    amount of biomass attached to the substratum; a general rule of thumb would be at
    least 10 tiles if biomass is low and 5 to 10 tiles if it is high).
2. Using a 30-mL automatic pipette, remove 30 mL of stream water from each
    chamber and transfer to the sample bottle labeled "initial". Filter the 30 mL water
    samples through a 0.45 μm pore size syringe filter (e.g., Syrfil-MF, Costar Corp.,
    Cambridge, MA). Start either the pumps or the stir bar in the chamber.
3. Remove 30 mL of stream water at 30 min and 60 min after the start of the
    incubation, and transfer the water to the appropriately labeled bottle. Filter the
    samples as in step 2. If the water samples are not going to be analyzed for soluble
    reactive phosphorus (SRP) within a few hours, place the bottles in the refrigerator
    (for storage up to 1 week) or freezer (for storage >1 week) until they can be
    analyzed for SRP levels (APHA *et al.* 1995; see following).
4. After 60 min, remove the tiles from the chamber. Place the tiles in an appropriately
    labeled aluminum weigh boat (etch the bottom of the boat with a sharp edge to
    designate sample number) and dry the tiles to constant weight at 105°C

(ca. 24–48 hr). Remove the weigh boats from the drying oven and transfer them to desiccators until weighing.
5. After the weigh boats have been weighed, place them in a muffle furnace at 500°C for at least 4 hr (make certain the ovens are at 500°C before timing), remove, and allow them to cool to room temperature in a desiccator, and reweigh. Calculate AFDM as the difference between the dry mass and the combusted mass.
6. Analyze water samples for soluble reactive phosphorus (see below).

4. SRP Analysis (from APHA *et al.* 1995)

1. Make up appropriate reagents:
   a. H_2SO_4 solution: Add 140 mL concentrated sulfuric acid to 900 mL of double distilled water.
   b. Ammonium molybdate solution: Dissolve 15 g of ammonium molybdate in 500 mL of double distilled water (store in polyethylene bottle in the dark).
   c. Ascorbic acid solution: Dissolve 2.7 g of ascorbic acid in 50 mL of double distilled water. Make immediately before using or keep frozen.
   d. Antimony potassium tartrate solution: Dissolve 0.34 g of antimony potassium tartrate in 250 mL double distilled water.
   e. Mixed reagent: Combine 25 mL of sulfuric acid solution, 10 mL of ammonium molybdate solution, 5 mL of antimony potassium tartrate solution, and 5 mL of ascorbic acid solution. Use within 6 hr of preparation.
   f. Phosphorus standards: (1) stock solution - Dissolve 0.2197 g of anhydrous K_2HPO_4 in 1 L of double distilled water ($1.00 \, mL = 50 \, \mu g \, P \, L^{-1}$); (2) Prepare four standard curve solutions by diluting from stock solution; (3) Develop a standard curve of absorbance vs. SRP concentration.
2. Add 3.0 mL of mixed reagent to 30 mL of standard and all samples, and mix thoroughly.
3. Wait for at least 20 min, but not longer than 1 hr, and measure absorbance of solution at 885 nm against distilled water on a spectrophotometer using 10-cm pathlength cuvettes (shorter pathlengths can be used, but this is not recommended as analytical sensitivity is reduced).
4. Calculate SRP concentration ($\mu g \, L^{-1}$) by comparing absorbance of sample against the standard curve.

5. Data Analysis

1. Plot the SRP concentration vs. time to determine whether or not the relationship appears to be linear. Calculate the net P uptake rate using the following formula:

$$V = \frac{([C_o - C_f] \times L)}{t} \tag{9.3}$$

where V = net uptake rate (μg P/hr), C_o = initial SRP concentration, C_f = final SRP concentration, L = incubation volume (in L), and t = time period of

| TABLE 9.3 | Sample Data Sheet for Determination of Net P Uptake. |

Stream	
Time (min)	SRP concentration ($\mu g\,L^{-1}$)
0	
30	
60	
Calculated uptake rate:	
Total AFDM or chl a in sample:	
Uptake per unit AFDM ($\mu g\,P\,mg\,AFDM^{-1}\,min^{-1}$):	
Uptake per unit Chl a ($\mu g\,P\,mg\,Chl\,a^{-1}\,min^{-1}$):	

incubation (hr). The net P uptake rate should then be normalized to either total biomass in the incubation (e.g., AFDM or chlorophyll a) or total substratum surface area. Use Table 9.3 for data entry and calculations.

D. Advanced Method 2: Phosphorus Radiotracer Method

(review cautionary notes on use of radioisotopes described previously)

1. Preparation Protocol

1. At least one month, and preferably 3–6 months, prior to the experiment, place small unglazed ceramic tiles or ceramic cylinders (Steinman *et al.* 1991b) in selected high-P and low-P streams to be sampled. If tiles are used, they should be pre-ashed to remove attached glue, which otherwise would be included in the AFDM measurement.
2. Label acid-washed Tupperware containers (30 cm × 30 cm) by stream name or type (high-P; low-P).
3. Each team should have six 25 mL scintillation vials, each containing 15 mL of Ecolume scintillation cocktail, labeled according to treatment (high-P or low-P) and time (background, 10, 20, 30, 45, and 60 min).

2. Field Collection Protocol

1. Collect tiles and water (filter 1 L of stream water into an acid-washed 1-L plastic bottle using a hand pump and a Whatman GFF or Gelman Type A/E glass-fiber filter) from each stream.
2. Place 10 to 16 (the latter if turnover is to be measured) tiles into labeled Tupperware containers (high-P or low-P), which are filled with stream water. Attach the lids to completely water-filled containers (which minimizes tile movement) and place containers in a cooler to be transported back to the laboratory.

3. Laboratory Protocol

1. Transfer the 1 L of filtered stream water and the tiles into each incubation chamber.[4]
2. Transfer approximately 50 mL of the filtered stream water to a 60 mL acid-washed plastic bottle, which will be analyzed for SRP concentration.
3. Remove 1 mL of water from each chamber just prior to the ^{33}P injection. Transfer this water to the appropriately labeled scintillation vial (background) and mix thoroughly.
4. Inject 0.5 mCi of carrier-free ^{33}PO$_4$ (as either orthophosphoric acid or phosphate salt dissolved in water) with a micropipette into each chamber. The micropipette tip will be extremely radioactive, so it should be removed immediately from the pipette after use and discarded in a radioactive waste bin.
5. Remove 1 mL of water from each chamber at 10, 20, 30, 45, and 60 min after the start of the incubation. Transfer the water to the appropriately labeled scintillation vial and mix thoroughly.[5]
6. After the 60 min sample is collected, carefully remove the tiles from the chamber using forceps or tongs. (**Remember: The material attached to the tiles will be radioactive and should be handled with great care.**) These tiles will then be processed for AFDM measurement, if turnover is not to be measured. For AFDM measurement, follow the procedures outlined below (step 7), making sure to avoid touching the radioactive material (keeping the tiles inside the beaker at all times minimizes this risk). If P turnover is to be measured, it is recommended that the tiles remain in the radiolabeled chamber water for an additional 5 hr (6 hr total) to allow for greater incorporation of ^{33}P by the algae. Radiolabeled tiles are then transported back out to the streams or to recirculating chambers/aquaria if turnover is to be measured. **Cautionary notes: Wear gloves, safety glasses, and lab coats at all times when handling radioactive samples (consult the local radiation safety officer at your institution for guidance and specific regulations associated with your site). Store the radioactive water from the incubation in sealed and labeled carboys until the radioactivity decays to background levels. It is recommended to store the water at least 10 half-lives before disposal (the half life of ^{33}P is 25.4 d).**
7. Finish measuring AFDM according to the methods outlined above, with the important modification of ***not*** removing the algae from the substratum. Simply weigh the substratum with attached algae, and calculate AFDM as the difference in mass before and after combustion. Double-bag, seal, label, and store the radioactive waste until the radioactivity decays to background levels.
8. Count each scintillation vial for 10 min on a liquid scintillation counter (the counting efficiency for ^{33}P is generally >90%, and because the sample matrix is the same for all samples, no correction for counting efficiency is needed). No decay correction is needed if all samples are counted within a few hours of each other.
9. Soluble reactive phosphorus concentration of the initial stream water will be measured according to the methods outlined above (Method C.4).

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[4] If there are obvious signs of seston in the chamber water, it will be necessary to filter the subsamples before they are added to the scintillation vials (to remove radioactively labeled particulate material). This can be done by removing approximately 3 mL of water from each chamber with a 15 mL syringe, filtering the water through a 0.45 μm pore size syringe filter (e.g., Syrfil-MF, Costar Corp., Cambridge, MA) into a small beaker, and then pipetting 1 mL of this filtrate into the scintillation vial.

[5] The first sample is not taken until 10 min to allow complete mixing of radioisotope within the chamber.

4. Data Analysis

1. Total phosphorus uptake rate is measured using the first-order rate coefficient of
    radiotracer depletion in the water (k), the concentration of SRP in the streamwater,
    and the water volume during the incubation (Steinman *et al.* 1991a). This
    procedure consists of three steps:

    a. Calculate k by regressing the ln-normalized scintillation count data (minus the
        background value determined from the sample collected just prior to ^{33}P
        injection) against time. Use Table 9.4 for data entry and calculations (also see
        Figure 9.4).
    b. Total P uptake rate is then estimated by multiplying k by the SRP
        concentration and by the water volume in the incubation chamber. Based
        on the data from the Sample Data Sheet and depicted in Figure 9.4, k
        (-0.0038/min $= -0.0228$/hr) is multiplied by 6.2 (SRP concentration)
        and 1.0 (L of water in chamber). This rate is in units of μg P/hr.
    c. Total P uptake rate should then be normalized to the biomass in the chamber
        (μg P mg AFDM^{-1} hr^{-1}) or by surface area of substrata in the chamber
        (μg P cm^{-2} hr^{-1}). The total uptake rate is divided either by the total AFDM in
        the chamber (e.g., 70.4 mg based on the Sample Data Sheet) or total substrata
        surface area in chamber (e.g., 160 cm^2 based on the Sample Data Sheet) to
        obtain a normalized uptake rate.

TABLE 9.4 Sample Data Sheet for Determination of Total Phosphorus (TP) Uptake (Radiotracer).

Time (min)	Counts per Minute (CPM ^{33}P)	CPM Minus Background	ln(CPM Minus Background)
Stream: Laboratory Stream with Grazing Snails			
0 (background)	49.4		
10	2009.8	1960.4	7.581
20	1939.1	1889.7	7.544
30	1841.7	1792.3	7.491
45	1761.8	1712.4	7.446
60	1669.4	1620	7.390

Calculated uptake rate constant (k): -0.0038/min

Streamwater SRP concentration (μg L^{-1}): 6.2

Calculated TP uptake rate (μg P min^{-1}): 0.024

AFDM (mg) or Chl *a* (mg) or surface area (cm^2) in sample:

  AFDM = 70.4 mg; surface area = 160 cm^2

Uptake per unit AFDM (μg P mg AFDM^{-1} min^{-1}): 0.000341

Uptake per unit Chl *a* (μg P mg Chl *a*$^{-1}$ min^{-1}):

Uptake per unit surface area (μg P cm^{-2} min^{-1}): 0.00015
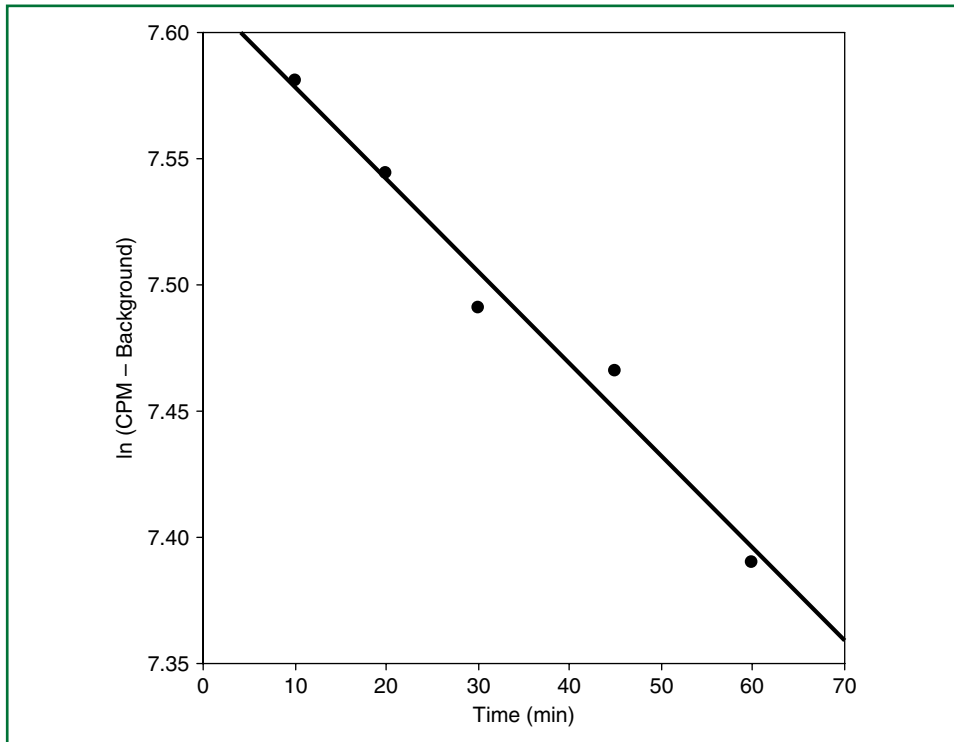
FIGURE 9.4 Radioactivity of ^{33}P in water incubated with periphyton exposed to grazing snails. Background activity of ^{33}P has been subtracted from measured activity and the data ln-normalized. Data used to generate the figure are based on real experiments and are included in the total P uptake sample data sheet.

E. Phosphorus Turnover

1. Preparation Protocol

1. Cover each of the 50 mL glass beakers with a square of aluminum foil and gently etch into the foil the following information: stream type (high-P or low-P), sampling date (Day 0, 2, 5, or 10), and replicate (a–d).

2. Field Placement and Collection (Option 1)

This option is to be followed if permission is obtained by the appropriate authorities to place radiolabeled material in the selected streams. If permission cannot be obtained, follow the steps in Method E.3 (Option 2, below).

1. After completion of the radioisotopic uptake study (Method D.6), carefully remove the tiles with forceps or tongs from each chamber, place them into Tupperware containers, and transport them to the high-P and low-P streams.
2. Place tiles in locations that have similar current velocities, irradiance levels, temperatures, and grazer densities in both streams, if possible. Remove 4 radioactive tiles from each stream at 1 hr (the one hour incubation allows "day 0"

samples to be rinsed by "cold" [i.e., nonradioactive] stream water prior to sampling in order to wash off adsorbed residual ^{33}P) and again at 2, 5, and 10 d, and place them in appropriately labeled, tared 50 mL glass beakers. One additional unlabeled tile should be processed from each stream; any radioactivity associated with the algae on this tile will be subtracted off all other counts, as it represents the naturally occurring background radioactivity (or the background activity associated with the scintillation counting of samples). The laboratory procedures associated with this option are included after Method E.3 (Option 2, below).

3. Chamber/Aquarium Placement and Collection (Option 2)

For this option, radiolabeled tiles are placed in two recirculating chambers or aquaria: one with a high-P and one with a low-P concentration (actual concentrations should mimic those of the natural streams that otherwise would have been used). Ideally, the water in each chamber should be changed daily to minimize released ^{33}P from being taken up again. However, this option is time-consuming and can generate a considerable volume of contaminated waste. If water is not changed, it should be recognized that the calculated turnover rates may represent underestimates of true turnover.

1. After completion of the radioisotopic uptake study (Method D.6), carefully remove the tiles with forceps or tongs from each chamber, place them into Tupperware containers, and transport them to the high-P and low-P chambers. Chambers should be exposed to similar light and temperature regimes and be fitted with a means to circulate or move the water (e.g., aeration or mixing).
2. Remove 4 tiles from each stream at 1 hr (the 1-hr incubation allows "day 0" samples to be rinsed by stream water prior to sampling in order to wash off adsorbed residual ^{33}P) and again at 2, 5, and 10 d, and place them in appropriately labeled, tared 50-mL glass beakers. One additional unlabeled tile also should be processed from each stream; any radioactivity associated with the algae on this tile will be subtracted off all other counts, as it represents the naturally occurring background radioactivity.

4. Laboratory Protocol (applicable for both Options 1 and 2)

1. On the day of collection (days 0, 2, 5, and 10), place the beakers in a drying oven and dry the tiles to constant weight at 105°C (usually 24–48 hr).
2. Once the tiles reach a constant dry mass, weigh the dried tiles, and combust them for a minimum of 4 hr at a full 500°C. Remove tiles, allow them to cool to room temperature in a desiccator, and reweigh.
3. Using a 10 mL pipette, add 10 mL of 2N HCl to the beaker (make sure this is enough to cover all the periphyton), label the beaker with the appropriate sample designation, and place parafilm over the beaker. Leaching of ashed material should last at least 24 hr. Place beakers in the laboratory hood.
4. After a minimum exposure of 24 hr to the acid, add 10 mL of distilled water to each beaker (to reduce acidity to 1N), swirl the beaker gently to mix thoroughly, and pipette 1 mL of the diluted leachate to a scintillation vial containing 15 mL of Ecolume scintillation cocktail.
5. Count each sample on a liquid scintillation counter for 10 min. It is recommended that all samples from the turnover experiment be counted during the same run

TABLE 9.5	Sample Data Sheet for Determination of Turnover.				
Stream					
ln(CPM)					
Replicate	Background	Day 0 Minus Background	Day 2 Minus Background	Day 5 Minus Background	Day 10 Minus Background
A					
B					
C					
D					

(within several hr of each other) at experiment's end to preclude the need to apply a radioactive decay correction factor. The counts are used to determine turnover rate.

5. Data Analysis

1. Phosphorus turnover rate is computed by linear regression of $\ln[^{33}P]$ counts in algae versus time in stream (in days). Phosphorus turnover rate is therefore expressed as a first-order turnover rate constant (d^{-1}). The background radioactivity associated with unlabeled tiles should be subtracted from each sample count prior to performing the ln-transformation and the regression, and the regression should be based on a mean value derived from the four substrata sampled on each day. Use Table 9.5 for data entry and calculations.
2. It should be emphasized that this determination of P turnover rate may not be an accurate physiological index of total algal P turnover rate because not all phosphorus pools within the algal cells will have reached isotopic equilibrium during the 6 hr of ^{33}P exposure during the uptake part of the experiment (cf. Scinto and Reddy 2003). However, the approach described should provide a reasonable basis for comparing turnover rates in the more rapidly cycling P pools between different streams.

IV. QUESTIONS

A. Limitation: Phosphatase Activity

1. Was phosphatase activity greater in the low phosphorus stream, as hypothesized? If not, what might explain this result?
2. What other factors besides phosphorus concentration and biomass might influence the PA in the two streams?
3. Why is it important to normalize the PA data by an index of biomass?
4. Phosphatase is an inducible enzyme. That is, it is synthesized upon metabolic demand, as opposed to a constitutive enzyme, which is always present. What advantage is there to an organism in maintaining phosphatase as an inducible enzyme?

B. Limitation: Chemical Composition

1. C:P ratios substantially greater than 106:1 (on a molar basis) suggest phosphorus deficiency in planktonic algae (the Redfield ratio). Why is the C:P ratio suggesting P deficiency different in plankton compared to benthic algae (>360:1)?
2. Some algal species have greater carbon demands than others because of more carbon-based compounds in their cell walls. How would this type of demand influence the interpretation of the C:P ratio?
3. Many algal species exhibit "luxury uptake" of phosphorus, whereby they take up excessive amounts of phosphorus when it is available (e.g., during high P conditions) and then store the P intra-cellularly (in polyphosphate bodies). How would luxury uptake of P influence the chemical composition ratio of benthic algae?

C. Net Uptake: Stable Phosphorus

1. Were the net uptake rates similar or different between the two streams? If they were different, what might account for this difference?
2. Sometimes, no net uptake is measured during an incubation (i.e., the amount of phosphorus measured at the start of the experiment is the same as that at the end of the experiment). Assuming that the algae are biologically active and actively taking up phosphorus, what might explain this result?
3. Would you expect the same P uptake rates by algae irrespective of which method of measuring uptake was used (i.e., stable vs. radioisotopic P)? If so, why? If not, how might the rates differ and why?

D. Total Uptake: Radiolabeled Phosphorus

1. Were the total uptake rates similar or different between the two streams? If they were different, what might account for this difference?
2. By keeping the incubation time short in this exercise, you minimize the possibility that any radioactive phosphorus that was taken up could be re-released within the incubation period (i.e., minimize the possibility of recycling). Thus, the radioactive phosphorus removed from the water is assumed to represent the total (gross) uptake rate. How does this differ from net uptake rate (i.e., which measure should be greater)? Why?

E. Turnover

1. Were the P turnover rates similar or different between the two streams? If they were different, what might account for this difference?
2. If the ^{33}P was allowed to come to complete isotopic equilibrium within the algae during the uptake part of the experiment, would you expect measured P turnover rates to be greater or lower than those measured? Why?
3. How might the thickness of the periphyton matrix influence turnover rates? What about grazing activity?

V. MATERIALS AND SUPPLIES

Letters in parentheses indicate in which Method (A, B, C, D, or E) the item is used.[6]

Field Materials

>   1-L polyethylene bottles (A, C, D)
>   Cooler (C, D)
>   Hand pumps with GFF (or equivalent) filters (A, C, D)
>   Holder to transport beakers to and from field (E)
>   Tupperware containers to accommodate tiles or rocks (A, B, C, D, E)
>   Unglazed ceramic tiles (e.g., tiles measuring 3×3 cm or ceramic cylinders)
>   (A, C, D, E)

Laboratory Materials

>   1N NaOH (A)
>   2N HCl (B, E)
>   10 mL glass beakers (B, D)
>   25-mL plastic syringes with syringe holders; 0.45 μm pore size (A, C, D)
>   25-mL scintillation vials (A, D, E)
>   50-mL glass beakers (E)
>   50-mL polyethylene bottles (C)
>   60-mL polyethylene bottles (D)
>   100 mL polyethylene bottles (B)
>   100-mL volumetric cylinders (B)
>   150 mM *para*-nitrophenyl phosphate solution (add 2.78 g of *para*-nitrophenyl
>   phosphate to 50 mL of double distilled water) (A)
>   90% acetone (90 parts acetone with 10 parts saturated magnesium carbonate
>   solution)
>   Aluminum foil (B, E)
>   Aluminum weigh boats (C, D)
>   Carrier-free ^{33}P isotope (0.5 mCi/chamber; order from New England Nuclear) (D)
>   Course-bristled toothbrushes (B)
>   Ecolume Scintillation Cocktail (ICN Scientific, Costa Mesa, CA) (D, E)
>   Large pans or trays (B)
>   Parafilm (B, E)
>   Plastic jars or centrifuge tubes (A)
>   Reagents for SRP analysis (B, D)
>   Wide mouth glass incubation jars (30 mL or larger for larger substrata) (A)

Laboratory Equipment

>   Analytical balance (B, C, D, E)
>   Automatic pipettes (1 mL; 10 mL; 30 mL) (A, B, C, D, E)
>   Cuvettes (1 and 10 cm pathlength) (A, B, C, D)
>   Desiccators (B, C)

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[6] Any use of isotope requires specific laboratory protocols. These protocols are available from the Safety and Health Department at the institution or laboratory licensed for isotope use. These protocols must be followed carefully. It is essential that gloves (we recommend double gloving, using vinyl gloves directly over the hands and disposable gloves over the vinyl ones), lab coats, and safety glasses be worn at all times.

Drying oven (B, C, D, E)
Liquid Scintillation Counter (D, E)
Muffle furnace (B, C, D, E)
Recirculating chambers (either with pumps or stirrers to circulate water) (C, D)
Spectrophotometer (narrow band width: 0.5 to 2.0 nm) (A, B, C, D)

VI. ACKNOWLEDGEMENTS

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Nitrogen Limitation and Uptake

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I. INTRODUCTION

A. Nitrogen as a Limiting Nutrient for Growth

All organisms need nutrients, such as nitrogen and phosphorus, for growth and reproduction. Nitrogen (N) circulates through the atmosphere and landscape in a complex cycle composed of biotic and abiotic transformations. The most abundant form, dinitrogen gas (N_2), composes nearly 78% of the atmosphere (Lutgens and Tarbuck 1992). Prior to human alteration of the global N cycle, the two mechanisms by which N gas entered the bioavailable N pool were biologically mediated N-fixation and, secondarily, via lightning. Because only a few specialized N-fixing organisms can directly use this gaseous pool, frequently N is a limiting nutrient in ecosystems (Vitousek and Howarth 1991).

Dissolved N concentrations in stream ecosystems are determined in part by watershed geology and vegetation (e.g., Gregory *et al.* 1991, Dodds 1997). Although stream flow continuously delivers dissolved N to biofilms (i.e., submerged colonized surfaces), N is often limiting to the algae, bacteria, and fungi that make up these biofilms (Pringle *et al.* 1986, Tank and Webster 1998, Francoeur *et al.* 1999, Wold and Hershey 1999, Tank and Dodds 2003). In addition to an adequate supply of N, organisms may be limited by the balance of nutrients available (e.g., nitrogen: phosphorus). A comprehensive understanding of the factors controlling N limitation and uptake in stream ecosystems is necessary to address problems associated with increased N loading in streams.

B. Increased Nitrogen Loading in Streams

Anthropogenic activities including burning fossil fuels, planting N-fixing crops, fertil-izer production, and wastewater disposal (Schlesinger 1997, David and Gentry 2000) have nearly doubled N inputs into the global cycle (Vitousek 1997). This excess N has converted once N-limited ecosystems to N-saturated ones (Fenn *et al.* 1998, Duff and Triska 2000, Bernot and Dodds 2005). Stream ecosystems are particularly threatened by anthropogenic increases of dissolved inorganic N, which enters mainly as ammo-nium (NH_4^+) in wastewater effluent (Duff and Triska 2000), and as nitrate (NO_3^-) in agricultural runoff (Howarth *et al.* 1996, David *et al.* 1997, David and Gentry 2000, Kemp and Dodds 2001). Increased N in stream ecosystems results in problems, including eutrophication and loss of species diversity, and in the human water supply can cause methylhemoglobinemia in infants and increased occurrence of non-Hodgkin's lymphoma (Vitousek *et al.* 1997, Carpenter *et al.* 1998, Rabalais *et al.* 2002). These problems rep-resent a loss of ecosystem health as well as a loss of economic goods and services. The ultimate fate of anthropogenic NH_4^+ and NO_3^- varies with differences in biotic demand (Duff and Triska 2000), abiotic sorption characteristics, substrata type (Kemp and Dodds 2002), and the physical retention characteristics of stream channels (Bernot and Dodds 2005). Continued research is needed to identify how N is used and retained within stream ecosystems.

C. Stream Nitrogen Uptake and Limitation: Consequences for N Retention in the Landscape

Nitrogen cycling in headwater streams has recently received attention due to the potential for small streams to process N and influence downstream export (Alexander *et al.* 2000, Sabater *et al.* 2000, Peterson *et al.* 2001). Mechanisms of N uptake in stream ecosystems include assimilatory uptake, denitrification, adsorption, burial, and volitalization (Bernot and Dodds 2005). In addition, the degree of N limitation influences stream N uptake capacity. Peterson *et al.* (2001) found that headwater streams are effective at removing and transforming dissolved inorganic N from the water column because of their high biological activity combined with increased sediment/water contact time. Because the uptake of dissolved inorganic N is especially high in shallow streams, these systems may play a key role in reducing N export to downstream ecosystems (Alexander *et al.* 2000). Stream biota can respond to increased N supply by increasing uptake and growth, but this response is limited by the processing capacity of the biota and physical factors such as light, temperature, and availability of other nutrients (e.g., phosphorus limitation). Eventually, with increased N loading, N uptake in streams will become saturated and excess N will be exported downstream (Bernot and Dodds 2005).

D. Overview of Chapter

Ecologists have described nutrient dynamics in streams by using the concept of uptake length, which is the average distance traveled by a dissolved nutrient before biotic uptake (Webster and Patten 1979, Newbold *et al.* 1981, Stream Solute Workshop 1990). By quantifying N uptake lengths, one can then calculate benthic demand for dissolved N as well as N removal rates from the water column per area of stream bed (Newbold *et al.* 1981). By measuring N limitation and uptake in streams, the demand and uptake efficiency of N relative to N supply within a stream ecosystem can be assessed.

In this chapter, we describe an easy method for determining if stream biofilms are N-limited using nutrient-diffusing substrata, followed by two different methods for quantifying whole-stream N uptake. The first describes a short-term N release, where N concentrations are elevated in the water column, and the decline in concentrations, relative to a conservative tracer, is measured downstream. Whole-stream N uptake reflects the apparent downstream decrease in dissolved inorganic N in the water column and represents the combination of N uptake from stream water and N remineralization back into stream water from biota. A more in-depth discussion of short-term solute dynamics can be found in Chapter 8 in this volume, which will complement methods described here.

The second uptake method we describe is a short-term stable isotope (^{15}N) tracer release, which keeps water-column N concentrations at ambient levels. Using this tracer method allows us to use the ^{15}N labeling of a food web compartment, like algae, to quantify whole-stream N uptake, while avoiding the difficulty of quantifying dissolved ^{15}N in the water column (Wollheim *et al.* 1999). In this case, whole-stream N uptake is represented by the ^{15}N that is assimilated into the biotic compartment and is reflected in the changing $\delta^{15}N$ values in their tissue. All three methods described in this chapter are complementary and, in combination, can provide assessment of N dynamics in one stream through time or comparison among streams. The objectives of these methods are to quantify N limitation and uptake in streams to gain a better understanding of the potential for streams to retain N in the landscape.

II. GENERAL DESIGN

A. Index of Limitation: Nutrient Diffusing Substrata

Nutrient diffusing substrata (NDS) provide a means for measuring whether the growth and/or activity of stream biofilms (autotrophic algae and heterotrophic bacteria and fungi) are nutrient-limited. *Basic Method 1* described below represents a modification from previously published work (Fairchild *et al.* 1985, Winterbourn 1990, Corkum 1996, Tank and Webster 1998, Tank and Dodds 2003). NDS are constructed using small plastic cups filled with nutrient-amended agar and topped with an inorganic surface (i.e., fritted glass disc) that allows for mainly algal colonization. Cups are then incubated in the stream for 18–20 days. Preliminary studies have shown that the rate of nutrient diffusion from the plastic cups, filled with agar and nutrient, is constant through 17 days and then declines only slightly to day 21 (J. L. Tank, unpublished data). Once inorganic surfaces have been colonized by algae, NDS are returned to the laboratory for analysis of algal colonization by measuring chlorophyll *a* standing stock. In the method below, we recommend constructing five replicates of each nutrient treatment as well as five replicate control NDS (no nutrient added to agar). Variability between NDS within a treatment can sometimes be high and five replicates allows for increased statistical power for determining treatment differences.

A two-factor analysis of variance (ANOVA), with presence of N and/or P as the main factors, is used to test whether algal biofilms are significantly affected by nutrient enrichment (Dube *et al.* 1997). Possible outcomes from the ANOVA on the bioassays are summarized in Table 10.1. Single nutrient limitation is indicated when just one of the nutrients (N or P) elicits a positive response, and the interaction term in the ANOVA is not significant. If neither N nor P alone significantly increase algal biomass ($p > 0.05$), but N and P added together (N+P) do (i.e., the interaction term in the ANOVA is significant; $p < 0.05$), then the algal biofilm is considered to be colimited by both N and P.

TABLE 10.1 Interpretation of NDS Responses to N and P Addition.			
Interpretation[a]	N effect	P effect	Interaction N × P
N limited	◆		
P limited		◆	
N and P colimited			◆
N and P colimited	◆	◆	
N and P colimited	◆	◆	◆
1° N limited, 2° P limited	◆		◆
1° P limited, 2° N limited		◆	◆
Not limited by N or P			

[a] *Diamond in N or P effect indicates a significant N or P limitation in the two-factor ANOVA ($p < 0.05$), and a diamond in the Interaction N × P indicates a significant interaction between the two treatments. (From Tank and Dodds (2003).)*

Similarly, there also could be colimitation by both N and P if, when added separately, they each stimulate algal biomass relative to controls, but the N and P responses are not different from each other. Secondary limitation is indicated if N or P alone significantly increases algal biomass, both N and P added together result in an even greater increase in biomass, and the interaction term for the ANOVA is significant.

Potential problems associated with the NDS method include loss of substrata due to floods or vandalism. We suggest using long, heavy stakes to secure the NDS bars to the stream benthos. If destruction of NDS by animals becomes a problem, cover NDS with a mesh cage for protection. In general, the NDS method provides a simple, cost-effective way to measure the nutrient status of algal biofilms and is generally highly successful. However, NDS only assess nutrient limitation or use by a singular stream compartment (i.e., algae colonizing inorganic substrata), whereas whole-stream nutrient uptake can be estimated using short-term nutrient enrichments (Basic Method 2) or short-term isotopic tracer additions (Advanced Method 1).

B. Short-Term Nitrogen Addition

Uptake of N can be measured using the short-term addition of a concentrated N solution (with a conservative tracer) dripped continuously into the stream to elevate concentrations of both N and the conservative tracer. When concentrations reach plateau (i.e., when in-stream concentrations reach a stable maximum) water samples are collected at stations downstream of the injection point and nitrogen uptake over the study reach can be calculated. To calculate an N uptake length (S_w), an exponential decay model is used:

$$\ln N_x = \ln N_0 - kx \qquad (10.1)$$
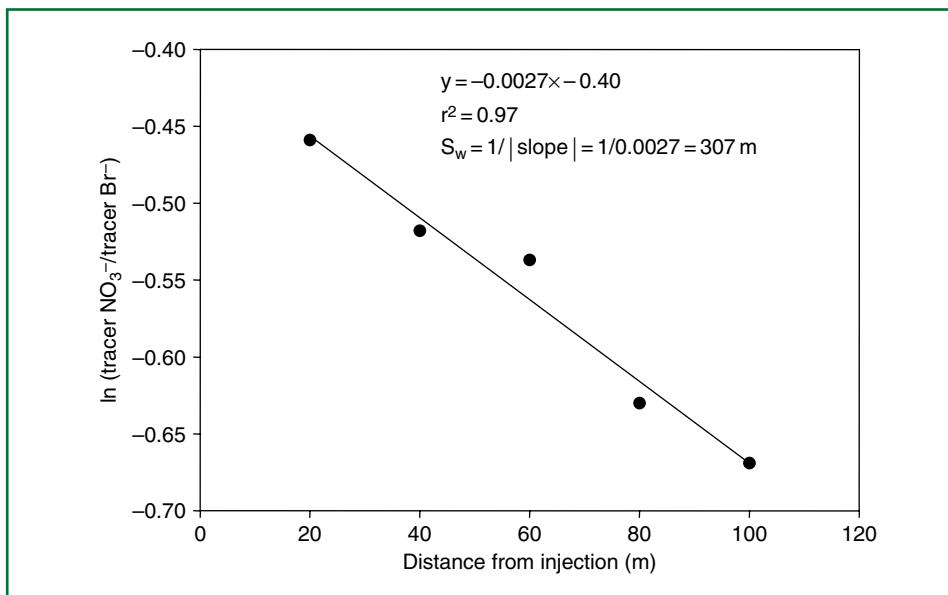
FIGURE 10.1 NO_3^- uptake length in Shane Creek, MI, in October 2003. Tracer NO_3^- and Br^- are plateau concentrations minus background concentrations. Uptake length, S_w, is the inverse of the slope of the regression line. Br^- is used as a conservative tracer to account for dilution along the stream reach.

where N_x is the background-corrected plateau N concentration at x meters downstream of the injection point, N_0 is the background-corrected N concentration at the site of injection, designated as 0 m, and k is the exponential decay rate (Figure 10.1; Newbold *et al.* 1981, Stream Solute Workshop 1990). A conservative tracer is added concurrently to account for dilution along the reach (see Chapter 8) and N concentrations are divided by plateau background-corrected tracer concentrations at each sampling station (Figure 10.1). A linear regression is then used to calculate the decay rate (k) and test for significance of the relationship. Uptake length (S_w) is calculated as the inverse of the decay rate:

$$S_w(\text{m}) = k^{-1} \tag{10.2}$$

Because uptake length is strongly influenced by discharge (Q), and Q may vary among streams or within a stream over time, N uptake velocity (V_f) should also be calculated. Nitrogen uptake velocity can be thought of as the biotic demand for N relative to in-stream concentration, and is functionally the velocity at which N is removed from the water column via uptake. Uptake velocity is calculated as:

$$V_f(\text{m min}^{-1}) = Qk/w \tag{10.3}$$

where Q is discharge ($m^3\,min^{-1}$) and w is mean stream wetted width. Precise estimates of Q can be made using the conservative tracer (see Chapter 8). The areal uptake rate (U, mg N $m^{-2}\,min^{-1}$) of N is calculated as:

$$U = V_f N_b \qquad\qquad (10.4)$$

where N_b is the background N concentration prior to release. When these parameters of N uptake (S_w, V_f, U) are used together, a greater understanding of the factors controlling uptake in stream ecosystems is possible, as uptake can then be understood relative to stream size, discharge, and nutrient availability.

Short-term N additions to streams have been used frequently due to the ease of studying multiple streams within a short period of time and the low cost of materials. Because NH_4^+ is one of the most labile forms of N in streams, downstream declines in concentration occur more quickly compared to NO_3^- or P additions. Thus, we describe an NH_4^+ release in *Basic Method 2*, although possible modifications to this exercise include changes in the form of the nutrient released (see Chapter 8). To ensure a decline in concentrations downstream when conducting a NO_3^- or P release, extend the length of the study reach and increase release duration. The NH_4^+ release described in *Basic Method 2* is conducted in conjunction with a chloride (Cl^-) conservative tracer release to correct for downstream dilution (see Chapter 8 for more detail). Chloride is the preferred conservative tracer, as increased chloride can easily be measured as the concurrent change in conductivity (using a field meter). However, if background water column conductivity in the study reach is high (such as in urban or agricultural streams), bromide (Br^-) can be substituted as the conservative tracer and measured using an ion-specific probe in the field or an ion chromatograph in the laboratory. Although there are drawbacks to using the amendments approach (see below), short-term nutrient releases are cost-effective and particularly useful for comparing uptake between streams or within one stream over time (Mulholland *et al.* 2002).

C. Short-Term ^{15}N Tracer Release

The goal of this method is to use a primary uptake compartment to quantify whole-stream N uptake by relying on the relative change in ^{15}N availability. In *Advanced Method 1*, we describe a short-term ^{15}N tracer addition where the longitudinal decay of label in a rapid-turnover compartment (e.g., filamentous green algae, epilithon, or organic sediments) is used as a surrogate for water-column ^{15}N label, and is sampled $\sim$6 hours after a continuous release of a ^{15}N enriched solution. A longer-term ^{15}N addition is described in Chapter 27, but the emphasis there is to interpret energy flow through the entire stream food web rather than uptake. However, these two methods may be combined to maximize knowledge of a stream ecosystem.

Stable isotope ^{15}N tracer additions eliminate the potential effects of N enrichment associated with short-term nutrient additions including the potential for artificially long uptake lengths due to saturation of N demand and/or stimulation of uptake in the presence of ample nutrient (Mulholland *et al.* 2002). However, the use of ^{15}N isotopes is more costly for both the short-term addition itself and subsequent required sample analyses via mass spectrometry. Using a rapid-turnover biomass compartment avoids the

difficult isotopic analysis of water samples. The biomass compartment will label with ^{15}N quickly so isotope additions of shorter duration can be used, thereby reducing isotope costs. Because the overall removal of ^{15}N from the water column will be reflected in the ^{15}N label in the biomass compartment, nitrogen uptake metrics (S_w, V_f, U) are calculated in the same way as for short-term releases of N. Substitute background-corrected $\delta^{15}N$ values of the biomass compartment at each station for water column N concentrations used in *Basic Method 2*.

If there are no fast-turnover compartments available within a study stream (e.g., a sandy-bottomed agricultural stream), an experimental compartment can be added to the stream prior to the short-term addition. These experimental compartments could include pre-colonized (~2 weeks) ceramic tiles or glass slides placed at the multiple sampling stations along the stream reach, thereby providing epilithon for sampling with the short-term ^{15}N tracer.

III. SPECIFIC METHODS

A. Basic Method 1: Nutrient Diffusing Substrata

Laboratory Procedures

General Laboratory Protocol
1. Make one agar type (control, $+N, +P, +N+P$) at a time.
2. See Table 10.2 for specific quantities of nutrient salts to add.
3. Polycon cups should be pre-labeled with agar type (with a Sharpie) on both sides and bottom of cup to ensure that the label remains legible during deployment in the stream.
4. Make a copy of Figure 10.2 for the NDS preparation checklist and gather items on the equipment checklist.

Agar Preparation (See Table 10.2)
1. Bring 1 L of water to boil and stir continuously with a 2.5-cm stir bar.
2. While stirring, add agar powder (2% by wt) and nutrients to water.
3. Bring solution to a boil and stir to prevent burning.
4. *Tips:*
   a. Solution is ready to pour when it becomes transparent.
   b. Bubbles will disappear as solution cools.
   c. Do not let the agar cool below 50°C or it will solidify.

Pouring Agar Solution
1. Carefully pour hot agar solution into cups until they are almost overfilled (rounded meniscus forms).
2. Allow 15 min for agar to cool and solidify. The agar will sink when cool.
3. Place fritted glass discs on agar surfaces and snap caps shut.
4. *Tips:*
   a. If lids don't close completely, carve out slight depression in agar for discs.
   b. Be sure that discs are held firmly in place and not loose under cap.

Date _____   Project _____

Stream _____   Investigators _____

(A) target nutrient	(B) salt A	(C) salt B	(D) molecular weight salt A	(E) molecular weight salt B	(F) desired molarity (M)	(H) g salt A per L agar solution	(I) g salt B per L agar solution	(J) g agar/L solution	(K) total number cups	(L) mL/cup	(M) total volume solution needed (L)
NH_4^+-N	NH_4Cl	—	53.5	—	0.5	26.7	—	20	5	30	0.150
NO_3^--N	KNO_3	—	101.1	—	0.5	50.6	—	20		30	
NO_3^--N	$NaNO_3$	—	85.0	—	0.5	42.5	—	20		30	
PO_4^{3-}-P	KH_2PO_4	—	136.1	—	0.5	68.0	—	20		30	
NH_4^+-N and PO_4^{3-}-P	NH_4Cl	KH_2PO_4	53.5	136.1	0.5	26.7	68.0	30		30	
NO_3^--N and PO_4^{3-}-P	KNO_3	KH_2PO_4	101.1	136.1	0.5	50.6	68.0	30		30	
NO_3^--N and PO_4^{3-}-P	$NaNO_3$	KH_2PO_4	85.0	136.1	0.5	42.5	68.0	30		30	
Calculation						D*F	E*F				(K*L)/1000
Explanation					0.5 M unless specific change			when adding two nutrients, an additional 10 g/L of agar should be dissolved into solution	generally 5 reps per treatment per stream	volume of agar solution to fill each Polycon cup	

Stream name _____ Date _____

Crew _____

Date NRS placed in field _____

Date NRS collected from field _____

NRS deployment locaton _____

NDS construction

❏ Purchase materials

❏ Drill openings in polycon cup lids

❏ Drill holes in L-bars

❏ Prepare agar w/nutrient
   treatments

❏ Fill Polycon cups with solution
   and allow to harden

❏ Place fritted glass discs on top of
   dried agar and close

❏ Secure Polycons to L-bar tightly
   with cable ties

❏ Place silicon beads on bottom
   of polycons to secure in place

❏ Cover L-bars with plastic wrap to
   keep moist and refrigerate

NDS deployment and collection

❏ Secure L-bars to benthos
   w/stakes at each end

❏ Incubate ~17 days

❏ Collect L-bars, remove discs and
   place in individually labeled Ziplocs

❏ Extract and measure concentrations
   chlorophyll *a* on discs

FIGURE 10.2 Data sheet and checklist for nutrient diffusing substrate construction, deployment in the field, and laboratory processing (Basic Method 1).

Attaching Cups to L-bars and Storage

1. Attach the cups to the L-bars using small cable ties (see Figure 10.3).
2. Secure each cup bottom to the L-bar using a small bead of rubber silicon glue.
3. Cut ends of cable ties close to the fastener to avoid sharp tips.
4. Cover each bar with plastic wrap to keep moist and refrigerate until stream
    deployment.
5. *Tips:*
    a. Polycon cups should be secured close to each other along the L-bars.
    b. Agar types (control, +N,+P,+N+P) should be randomly arranged among the
        L-bars.
    c. Tighten cable-ties with needle-nose pliers.

Field Procedures

Placement of NDS in Stream (See Figure 10.3)

1. In the stream, place the L-bars next to each other in a riffle.
2. Position the bars parallel to flow to help prevent sedimentation.
3. Pound stakes into holes at each end of the L-bar to secure into stream bottom.
4. If you can, check on NDS over 18–20 day incubation period.
5. *Tip:* If some NDS are lost within the first 48 h, they can be replaced but make sure
    to note difference in incubation time.
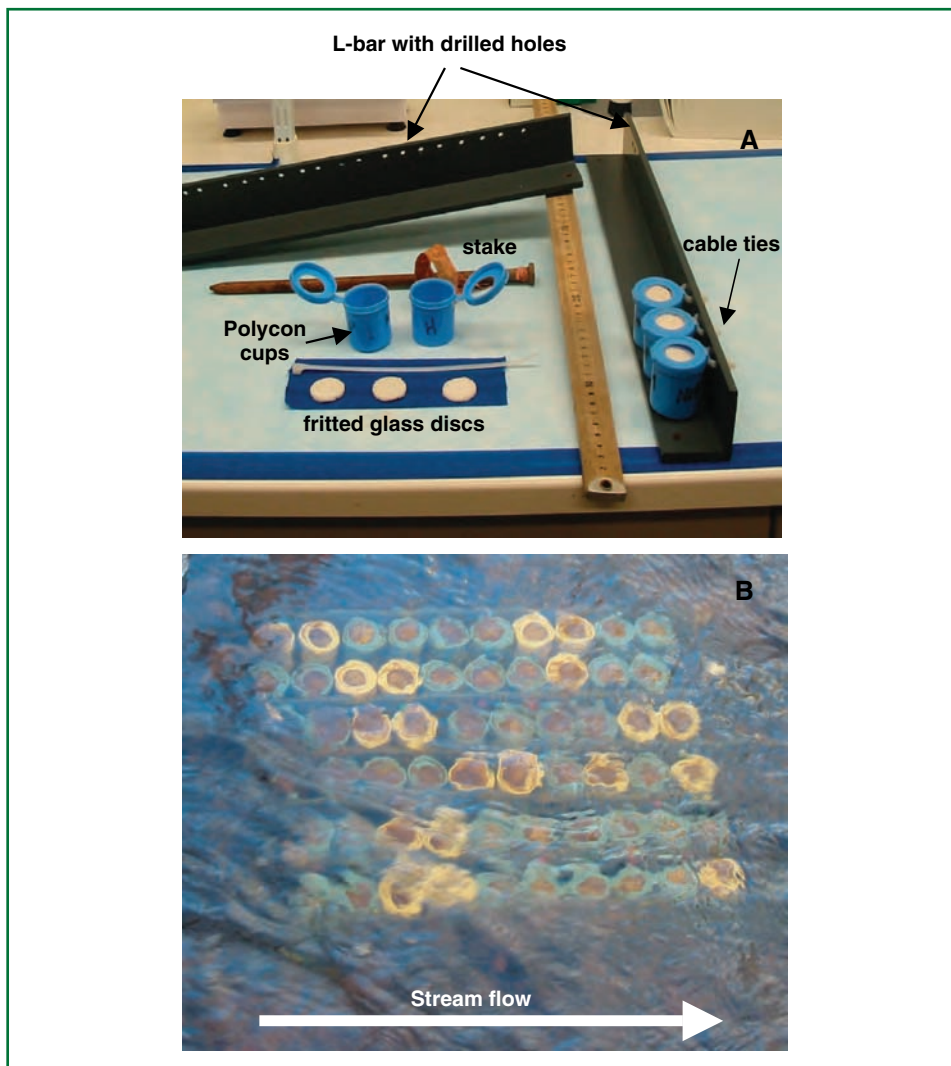
FIGURE 10.3 (A) Equipment needed for preparation of nutrient diffusing substrata (NDS) and (B) stream placement of NDS parallel to stream flow.

Retrieval from Stream

1. After 18–20 days, gently remove one L-bar at a time from stream.
2. Uncap each cup and immediately place each disc into a labeled Ziploc® baggie using forceps and place on ice in the dark until analyzed for chlorophyll *a*.

Chlorophyll *a* Analysis on Discs

1. Extract chlorophyll *a* directly from discs by placing each disc in a labeled film canister and covering disc with acetone (normally 10 or 15 mL). See Chapter 17 in this volume for details on chlorophyll extraction and analysis.
2. Express chlorophyll *a* per unit surface area and compare across treatments using two-factor ANOVA (Tank and Dodds 2003).

B. Basic Method 2: Short-term Nitrogen Release

General Preparations

Site Selection and Solute Decisions

1. Select an appropriate stream reach for the release. The stream reach should have minimal inputs from groundwater sources or point discharges. Groundwater inputs can be detected by identifying changes in water temperature or by conducting a conservative tracer release prior to the short-term N release. The selected stream should be relatively homogenous (i.e., no large changes in structure or composition) over the entire experimental reach.

2. Before going into the field, determine the quantity of N and conservative tracer salts needed for the injectate, keep the following relationship in mind:

$$Q_I = Q * C_s / C_I \tag{10.5}$$

where Q is stream discharge, C_s is the background N concentration, Q_I is the injectate drip rate into the stream, and C_I is the N concentration in the carboy (see Chapter 8).

3. For precise measurements, use a recent estimate of background N concentrations and stream discharge.

4. *Tips:*

    a. If you are unsure of the stream discharge (i.e., there is no recent estimate available), weigh salts in increments needed for $10\,\text{L s}^{-1}$ of stream discharge. You can add salts accordingly once you arrive at stream and measure stream discharge (see Chapter 3 for methods).

    b. If not enough salt is available, the drip rate can be altered. For example, you can either use X amount of salt and a drip rate of $0.1\,\text{L min}^{-1}$ or you can use half of X amount of salt and a drip rate of $0.2\,\text{L min}^{-1}$. Make sure to check the total volume needed for the release to ensure you have enough injectate for the duration of the release.

Calculating the Amount of Salt to be Added to the Carboy (See Table 10.3)

1. The quantity of salt added can be calculated as follows (using NH_4Cl as an example):

$$\text{g as NH}_4\text{Cl} = \overbrace{\left(\frac{\text{L}}{\text{sec}}\right)}^{Q} * \overbrace{\left(\frac{\mu\text{g N}}{\text{L}}\right)}^{\substack{\text{Target}\\\text{addition}}} * \overbrace{\left(\frac{\text{min}}{\text{L}}\right)}^{\frac{1}{\text{drip rate}}} * \overbrace{\left(\frac{20\,\text{L}}{1}\right)}^{\substack{\text{L in}\\\text{carboy}}}$$

$$* \underbrace{\left(\frac{\text{NH}_4\text{Cl mol wt. }53.49}{\text{N mol. wt }14}\right)}_{\text{N in NH}_4\text{Cl}} * \underbrace{\left(\frac{60\,\text{sec}}{\text{min}}\right) * \left(\frac{1\,\text{g}}{1000000\,\mu\text{g}}\right)}_{\text{conversions}} \tag{10.6}$$

2. *Tip:* Optimal target additions should be just high enough above background to be analytically detectable at the most downstream site, but not so high at the top site so that N demand is saturated (see Table 10.3 for examples).

Laboratory Preparations for the Field

1. Choose 5 sampling stations spaced approximately evenly downstream of the injection site (e.g., 20, 40, 60, 80, and 100 m from injection point).
2. For each station, label four clean 60 mL wide-mouth bottles, one for background (BKD) N samples and three for plateau (PLT) N samples.
3. Place the bottles for each station (1 BKD and 3 PLT) in separate Ziploc®.
4. Also, label one 60 mL bottle "CAUTION: RELEASE SOLUTION" and place in a separate bag.
5. Make a copy of Figure 10.4 for a field data sheet and gather items on checklist.

Field Procedures

Before Turning on the Dripper

1. Mark sampling stations with flagging tape, using a meter tape to measure distances, and set bottles at each station.
2. Collect BKD water samples and measure conductivity at each station.
3. *Tips:*
   a. Take care not to disturb the stream bed before and during release.
   b. Water samples can be filtered in the field using a syringe-mounted 25-mm filters or back in the laboratory prior to water chemistry analyses. Water samples should be frozen until N analysis can be performed.
   c. If background conductivity is too high, you will need to use Br^- instead of Cl^- as a conservative tracer during the release. Bromide concentrations can be measured with an ion-specific probe to detect plateau. If no ion-specific probe is available, calculate travel time and estimate the time to plateau. Collected water samples for N concentration (BKD and PLT) will also need to be analyzed for Br^- concentrations.

Preparing Injectate

1. Fill a 20-L carboy with 18 L of stream water, pour the pre-weighed salts into the carboy, and mix well to fully dissolve all the salts. Bring the volume to 20 L. Both N and conservative tracer salts should be added to the carboy.
2. Record the initial and final volume of the carboy before and after the N release so you can calculate the volume you injected over the release.
3. *Tip:* Use caution with injectate as this is a highly concentrated salt solution. Be careful not to splash injectate solution into any sample bottles or spill any into the stream.

Adding Injectate to Stream and Sampling at Plateau

1. Set up the pump and a place for the tubing to drip the injectate into the stream. It may be useful to cable-tie the tubing to a stake and secure into benthos, suspending the tubing slightly above water surface.
2. Place inlet and outlet of pump ends into the stream to fill tubing with stream water and rinse pump.

TABLE 10.3 Basic Method 2: Calculation of Solute to Add to Carboy for Short-term Nutrient Releases.

Date _____   Project _____

Stream _____   Investigators _____

(A) target solute	(B) background concentration (µg/L)	(C) Q (L/s)	(D) Enrichment concentration (µg/L)	(E) drip rate (L/min)	(F) Salt	(G)[a] proportion target solute:salt	(H) carboy volume (L)	(I) salt to add to carboy (g)	(J)[b] maximum solubility at 10°C (g/L)
Br^-	<10	10	40	0.1	NaBr	0.776	20	6.2	459
Cl^-	10	10	1000	0.1	NaCl	0.603	20	197.7	358
NH_4^+ -N	10	10	20	0.1	NH_4Cl	0.260	20	9.2	333
PO_4^{3-} -P	10	10	20	0.1	NaH_2PO_4	0.258	20	9.2	699
NO_3^- -N	150	10	50	0.1	$NaNO_3$	0.165	20	36.4	209
Calculation								(C*D*H*60) (G*E*1000000)	

[a] Molecular weight of target solute divided by molecular weight of salt, e.g., molecular weight of N divided by molecular weight of NH_4Cl.
[b] If g salt/L exceeds maximum solubility, then salt will not dissolve. Try increasing drip rate and carboy volume.

Stream name_____ Date_____

Crew_____

Solute_____ Injectate release rate checks _____

Start_____   _____

Stop_____ Injectate sample collected

Min_____

Field checklist

Station	Distance from injection (m)	BKD Conductivity	PLT Conductivity
1			
2			
3			
4			
5			

❏ Collect BKD water samples and BKD conductivity at all stations
❏ Turn pump on
❏ Set injectate release rate
❏ Start NH_4^+/Cl^- release
❏ Check injectate release rate
❏ Get PLT NH_4^+ samples and conductivity at all stations
❏ Check injectate release rate again
❏ Collect sample of injectate
❏ Turn pump off
❏ Measure stream width at 5–10 m intervals

FIGURE 10.4   Data sheet and checklist for short-term nutrient releases (Basic Method 2).

3. Test drip rate by placing the outlet tubing into a small graduated cylinder for 15 sec. Multiply volume of water collected by 4 to determine drip rate ($mL\,min^{-1}$). Record drip rate in field book.

4. Place weighted inlet tubing into carboy and remove air bubbles in tubing.

5. Start pumping injectate into the stream and record the exact time you begin the release (i.e., start stopwatch).

6. At the downstream station, monitor the rise in conductivity and wait for plateau. Conductivity will increase as the injectate reaches the downstream station. Plateau has been reached when conductivity no longer increases but remains stable.

7. Once conductivity stabilizes, take PLT water samples and PLT conductivity readings at each station moving from downstream to upstream.

8. Measure the injectate drip rate again and record in field book. Make sure to empty injectate from graduated cylinder into the stream. Turn off the pump and record the exact stop time and total release minutes, as well as the final volume of injectate in the carboy.

9. Collect a sample of the injectate and put into labeled bottle. Keep this bottle away from other samples to avoid contamination.

10. Place water samples in a cooler for transport to the laboratory; upon return place all samples into a freezer until ready for N analyses.

11. Collect data on stream widths after release is completed. Stream widths should be measured every 5–10 m from the injection point for ~20 measurements. Width measurements will be used to calculate N uptake parameters.

12. *Tips:*
    a. Wear gloves and use care when handling injectate to avoid contamination.
    b. It is very important that there be complete mixing of the injection by the first sampling station. Dripping injectate into a riffle area (either natural or constructed) will help with mixing.
    c. Rinse the pump well after releases because salt solutions are highly corrosive. Pull weighted inlet tube out of carboy and place just upstream of the drip point and rinse tubing thoroughly (~5 min) with clean stream water.

Laboratory Procedures

Analysis of Water Samples

1. Analyze water samples for NH_4^+-N concentrations using standard methods (APHA 1995). If using Br^- as a conservative tracer, analyze those samples as well.
2. *Tips:*
    a. When analyzing water samples, be sure to analyze your samples from low to high concentrations to avoid contamination (i.e., run BKD samples first, then PLT samples moving from downstream samples toward injection point).
    b. Two methods currently exist for analyzing NH_4^+-N: the indophenol blue method for samples above $10 \, \mu g \, L^{-1}$ (APHA 1995) and the fluorometric method for low concentrations (Holmes *et al.* 1999). When choosing an appropriate method, make sure to check the most recent literature for any refinements of the ammonium method.

Calculating N Uptake Length (S_w)

1. Enter all BKD and PLT concentrations measured for each station into a spreadsheet and calculate the mean PLT concentration at each station.
2. Plot ln(mean PLT-BKD NH_4^+-N)/(PLT-BKD conductivity) versus distance downstream (see Figure 10.1).
3. Using the regression equation for the plotted line, S_w (m) is the inverse of the absolute value of the slope.
4. Calculate uptake velocity, V_f, and whole-stream uptake, U, using S_w (see General Design section).

C. Advanced Method 1: Short-Term ^{15}N Tracer Release
General Preparations

Isotope Purchase and Site Selection

1. Purchase the stable isotope well in advance of your release (~3–6 months). We recommend a target enrichment for the stream of $\delta^{15}N = 5000$ ‰. If cost is an issue, target enrichment can be decreased, but reduced target enrichment results in less label incorporation in the selected biomass compartment.
2. Also, find an analytical laboratory that can conduct the mass spectrometry on organic solid samples, either at your own institution or one that does contract work. Check the web and see information below (see also Chapter 27).
3. Select your stream reach using the same criteria as for Basic Method 2, bearing in mind that isotope costs are directly proportional to stream discharge and N concentrations (i.e., NO_3^- flux). Small or low-N streams are the most cost-effective.

Pre-sampling and Preliminary Information for Calculations
1. Collect preaddition water samples and discharge measurements from a minimum of 5 points along the length of the stream reach. Because these data will be used to calculate how much isotope to add, precise measurements of N concentration and discharge are imperative.
2. See Table 10.4 for calculation of ^{15}N needed. Amount of ^{15}N to add to the stream channel is based on known variables including stream discharge, stream NO_3^- concentration, desired enrichment, enrichment of isotope salt, and experiment duration. Thus, these variables should be determined prior to calculation of ^{15}N needed. It is assumed that the amount of ^{15}N added to the carboy is the amount needed for the entire release. Drip rate of the pump should be adjusted so solute is almost completely depleted over the course of the release and should be calculated prior to the release.
3. See Basic Method 2, Table 10.3 for calculation of conservative tracer salt needed. To calculate isotope needed, first calculate the background ^{15}N flux:

$$^{15}N \text{ flux (g/d)} = \overbrace{\left(\frac{L}{sec}\right)}^{Q} * \overbrace{\left(\frac{\mu g\ N}{L}\right)}^{NO_3\text{-}N}$$

$$* \overbrace{\left(\frac{60\ sec}{min}\right) * \left(\frac{60\ min}{h}\right) * \left(\frac{24\ h}{d}\right) * \left(\frac{g}{1000000\ \mu g}\right)}^{conversions} * \overbrace{\left(\frac{1\ ^{15}N}{273\ ^{14}N}\right)}^{^{15}N\ ratio}$$

$$(10.7)$$

4. To calculate g $K^{15}NO_3$ to add to 20-L carboy, incorporate the calculated ^{15}N flux into the equation below:

$$\begin{array}{c} g\ K^{15}NO_3 \\ \text{to add to} \\ \text{20L carboy} \end{array} = \left(\left(\left(\overbrace{\left(\frac{g}{d}\right)}^{^{15}N\ flux} * \overbrace{\left(1+\left(\frac{5000}{1000}\right)\right)}^{\substack{target \\ enrichment}}\right)\right) - \overbrace{\left(\frac{g}{d}\right)}^{^{15}N\ flux}\right) * \overbrace{\left(\frac{d}{24\ h}\right)}^{convert}$$

$$\overbrace{* (6\ h)}^{duration} \bigg/ \left(\left(\overbrace{\left(\frac{^{15}N}{^{14}N}\right)}^{\substack{enrichment \\ of\ K^{15}NO_3}} * \overbrace{\left(\frac{14\ g\ N/mol}{101.2\ g\ KNO_3/mol}\right)}^{ratio\ of\ N\ in\ KNO_3}\right)\right) \qquad (10.8)$$

5. Note that the target enrichment of 5000 and the duration of the release of 6 hrs are suggested values and can be modified to suit specific conditions.
6. *Tips:*
   a. After you calculate the quantity of isotope and conservative tracer (Cl^- or Br^-) needed, weigh into containers that are well-sealed to avoid contamination.

TABLE 10.4

Advanced Method 1: Calculation of ^{15}N-Labeled Salt to Add to Stream Channel Over the Course of the Experiment.

Date _____   Project _____

Stream _____   Investigators _____

	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)	(J)	(K)[a]	(L)	(M)[b]
stream	discharge (L/s)	NO_3^--N (μg/L)	target $\delta^{15}N$ (‰)	NO_3^--N Flux (μg/d)	NO_3^--N Flux (g/d)	^{15}N Flux (g/d)	^{15}N needed for target (g/d)	total ^{15}N to add (g/h)	drip duration (h)	total ^{15}N needed for release (g)	enrichment of K^{15}NO$_3$	total K^{15}NO$_3$ needed for release (g)	cost (US$)
A	10	500	5000	432000000	432	1.58	9.50	0.33	6.00	1.98	0.10	143	1000
B	10	1000	5000	864000000	864	3.16	18.99	0.66	6.00	3.96	0.10	286	2000
C	10	1000	5000	864000000	864	3.16	18.99	0.66	6.00	3.96	0.99	29	2400
D	50	1000	5000	4320000000	4320	15.82	94.95	3.30	6.00	19.78	0.99	144	12000
Calculation				A*B*60*60*24	D/1000000	E/273	F*(1+(C/1000))	(G-F)/24		I*H		J/(K*(14/101.2))	
Explanation				convert to days	convert to grams	multiply by background ratio of ^{15}N:^{14}N	calculating ^{15}N needed for target enrichment	subtract ^{15}N natural abundance and convert to hrs		multiply g^{15}N/h by drip duration	proportion of ^{15}N to ^{14}N in KNO$_3$	divide total ^{15}N (g/h) by ratio of molecular weight N:KNO3 multiplied enrichment of K^{15}NO$_3$	multiply g needed by cost

[a] Isotope (e.g., K^{15}NO$_3$) can be purchased in various degrees of ^{15}N enrichment (10% and 99% ^{15}N-enriched are most common but be sure to check isotope bottle label).

[b] Costs are based on 2006 estimate of $80 per gram of 99% enriched and $7 per gram 10% enriched K^{15}NO$_3$. Note that you can use more 99% enriched K^{15}NO$_3$ without changing background nitrate concentration.

b. Because of the potential for contamination, all salts weighed out should be clearly labeled and double-bagged.

c. Be extremely careful when weighing out isotope salts. THIS SHOULD BE DONE AWAY FROM ANY SAMPLE PROCESSING. Even a miniscule amount of salt can contaminate an entire laboratory and ruin all current and future experiments where isotopes would be used!

d. Prepare an area of the laboratory designated for isotope sample processing. Clear area, wipe down with ethanol, and lay down laboratory paper. Signs should be placed around area indicating that enriched samples are being processed.

Field Procedures

Before Turning on the Dripper

1. Measure a 200–300 m stream reach and mark 10 stations along the reach with flagging tape (if $Q > 50\,L\,s^{-1}$, extend stream reach and mark additional stations).

2. Choose stations based on expected uptake (e.g., if the stream is inundated with algae, uptake will be high and stations should be concentrated closer to the injection point with only a couple further downstream).

3. Collect background samples of your rapid-turnover compartment (i.e., filamentous green algae, biofilm, or organic sediments) at each sampling station for determination of the natural abundance $\delta^{15}N$ signal. See below for laboratory processing details.

4. Make a copy of Figure 10.5 for the field data sheet and gather items on checklist.

Stream name_____ Date_____
Crew_____
Injectate release rate checks_____
Start time_____ g Cl⁻ added_____
Stop time_____ g $K^{15}NO_3$ added_____
Total drip time (min)_____Carboy volume_____

Station	Distance from injection (m)	BKD Conductivity	PLT Conductivity
1			
2			
3			
4			
5			

Field checklist
- ❑ Collect BKD compartment and conductivity samples at all stations
- ❑ Turn pump on
- ❑ Set injectate release rate
- ❑ Start ^{15}N/Cl⁻ release
- ❑ Check injectate release rate
- ❑ Collect PLT compartment and conductivity samples at all stations
- ❑ Check injectate release rate again
- ❑ Turn pump off
- ❑ Rinse samples thoroughly
- ❑ Measure stream width at 5–10 m intervals

FIGURE 10.5 Data sheet and checklist for short-term ^{15}N tracer release (Advanced Method 1).

Carboy Preparation

1. Fill carboy with ∼18 L of stream water and set up pump at injection point.
2. While wearing gloves, add a small amount of water to the vial containing the ^{15}N salt to dissolve just prior to adding it to the carboy.
3. While wearing gloves, add isotope and conservative tracer salt to carboy. Rinse vials a few times into carboy after addition to remove all of the salts.
4. While wearing gloves, mix injectate by capping and shaking carboy or stirring with a stick after bringing volume to 20 L.
5. *Tips:*
   a. Record the mass of $K^{15}NO_3$ salt added to the injection carboy and the initial volume of injectate.
   b. Be sure to wear disposable gloves when handling the ^{15}N salt or solution and carefully discard the gloves when finished. Contamination by ^{15}N is a major concern.

Adding Injectate to Stream and Sampling at Plateau

1. Suspend tubing in center of stream channel slightly above surface keeping rest of the tubing out of the stream. Drip injectate into stream at a constant rate (rate should be set so that 20-L carboy is almost completely emptied over the duration of the experiment) for ∼6 h in order to label the rapid-turnover compartment with ^{15}N.
2. Record the start time the injection began and pump flow rate (determined using timed drip into graduated cylinder; see Basic Method 2 for details). Make sure to empty injectate from graduated cylinder into the stream.
3. After 6 h of continuous injection, collect samples of your fast-turnover compartment (e.g., algae) at each station (moving from downstream to upstream stations) for later ^{15}N analysis. Make sure you have a sample from before the drip begins for background stable isotope analysis of biomass compartment.
4. Place samples in small Ziploc® and label clearly with date, stream, site, and "^{15}N sample."
5. At the same time, to determine the effects of dilution on isotope labeling, take water samples for conservative tracer concentrations (as described in Basic Method 2 above) or measure conductivity at each station.
6. After all plateau isotope and conservative tracer samples have been collected, turn off injection pump. Rinse tubing thoroughly by pumping clean water from upstream of the injection point for ∼10 min.
7. Record the final volume of injectate in field book.
8. To avoid contamination of biomass compartment samples by the ^{15}N in the water column when you sampled, go upstream of dripper and rinse samples thoroughly before placing samples on ice for transport back to the laboratory. Rinse samples starting with lowest enrichment, those taken from the most downstream site, and process moving upstream to avoid contamination.
9. Collect data on stream widths after release is completed. Stream widths should be measured every 5–10 m from the injection point for ∼20 measurements. Width measurements will be used to calculate uptake parameters.
10. *Tips:*
    a. Check the injection rate every hour. After a rate check, be sure to put graduated cylinder contents into the stream. Adjust injection rate if necessary.

b. It is very important that there be complete mixing of the injection by the first sampling station. Dripping injectate into a riffle area (either natural or constructed) will help with mixing.

c. A composite sample of each compartment should be collected at each station. For example, take a little bit of algae from several places at each station and combine for ^{15}N sample.

Laboratory Procedures

Sample Processing for Rapid-Turnover Compartments

1. To avoid contamination, sort compartment samples from lowest to highest enrichment for processing (i.e., downstream to upstream).
2. Place each sample into sieve and thoroughly rinse off with tap water to ensure no enriched water dries onto the sample. Continue until all samples have been thoroughly rinsed. Dry all equipment and sample bags thoroughly after rinsing.
3. Place each sample into labeled aluminum pan for drying.
4. Dry compartment samples at 55° C for 72 h.
5. Grind samples into a fine powder and place into labeled scintillation vials for storage until analysis via mass spectrometry.
6. Prepare each sample for analysis of ^{15}N by mass spectrometry by weighing a small amount of ground sample (using a microbalance) into a 4.6-mm tin capsule (Costech Laboratories) and then press into a sealed "bullet shaped" capsule.
7. Analyze samples on a mass spectrometer, using a reputable university or commercial analytical laboratory capable of analyzing ^{15}N samples.
8. *Tips:*
   a. Thoroughly rinse ALL field equipment with tap water to ensure that no ^{15}N contaminates the laboratory.
   b. Keep all ^{15}N contaminated materials and samples separate and clear of other equipment in the laboratory.
   c. Prepared sample capsules can be placed into a 96-well plate and covered with a well cap for transport to mass spectrometry laboratory.
   d. If cost is inhibiting, analyze samples from every other station (i.e., stations 1, 3, 5, etc.) first to determine where peak ^{15}N enrichment occurred and then analyze more samples if necessary.
   e. Ground ^{15}N samples in scintillation vials will contain enough material for multiple analytical replicates if needed.

Potential Modifications to Protocols

1. Either $^{15}NH_4^+$ or $^{15}NO_3^-$ can be used as an isotopic tracer.
2. In areas with high background streamwater conductivity, Br^- should be used as the conservative tracer instead of Cl^-. Bromide can be measured using an ion-specific probe or water samples can be analyzed on an ion chromatograph in the laboratory (see details in Basic Method 2).
3. If the selected stream reach does not have an abundance of filamentous green algae, alternative substrata can be used for the rapid turnover compartment including bacterial biofilm and flocculent fine benthic organic matter. But these compartments may have lower N uptake rates, resulting in slower incorporation of

the ^{15}N tracer. The more active the compartment (i.e., rapidly cycling), the more isotopic tracer will be incorporated, resulting in a higher tracer $\delta^{15}N$ signal.

4. If your stream is depleted of biota, unglazed ceramic tiles can be colonized ahead of time with stream biofilm by placing them in the stream 2-4 weeks prior to the short-term isotope addition. Tiles are then harvested after the 6-h isotope release and analyzed in the same manner as previously described.

Calculation of ^{15}N Uptake Length

1. Uptake length is calculated similarly as in Basic Method 2, Equation 10.2. However, ^{15}N uptake length (S_w) is calculated using the inverse of the slope of the regression of ln(tracer $\delta^{15}N$ compartment/conservative tracer concentration) versus distance from the injection point in meters (see Figure 10.6). "Tracer" is defined in Figure 10.1. Uptake velocity (V_f) and whole-stream uptake (U) are then calculated as described above (Equations 10.3, 10.4).

IV. QUESTIONS

Basic Method 1: Nutrient Diffusing Substrata

1. Was each stream limited by N or P? Did colimitation occur? What factors may explain your results?
2. When would you expect N limitation to occur? When would you expect P limitation to occur? When would you expect colimitation to occur?
3. Why is it important to understand what nutrients limit algal growth in streams?

Basic Method 2: Short-Term Nitrogen Release

1. What was the N uptake length (S_w), uptake velocity (V_f), and uptake rate (U) calculated for each stream?
2. How do spiraling metrics compare to other stream ecosystems? How does areal uptake compare to terrestrial ecosystems?
3. Would you expect NO_3^- or NH_4^+ uptake rates to be higher? How would P uptake compare?

Advanced Method 1: Short-Term ^{15}N Tracer Release

1. What was the N uptake length (S_w), uptake velocity (V_f), and uptake rate (U) calculated for each stream?
2. How would N uptake compare if different biotic compartments were sampled for ^{15}N enrichment? Would the estimate be similar? Why?
3. How did the N uptake rate determined using Basic Method 2, compare to the N uptake rate determined using Advanced Method 1? Are patterns similar for S_w and V_f?
4. Sometimes N uptake cannot be detected within a stream reach using either a short-term N release or a short-term ^{15}N tracer release. What factors would yield these results for each method?

A

B
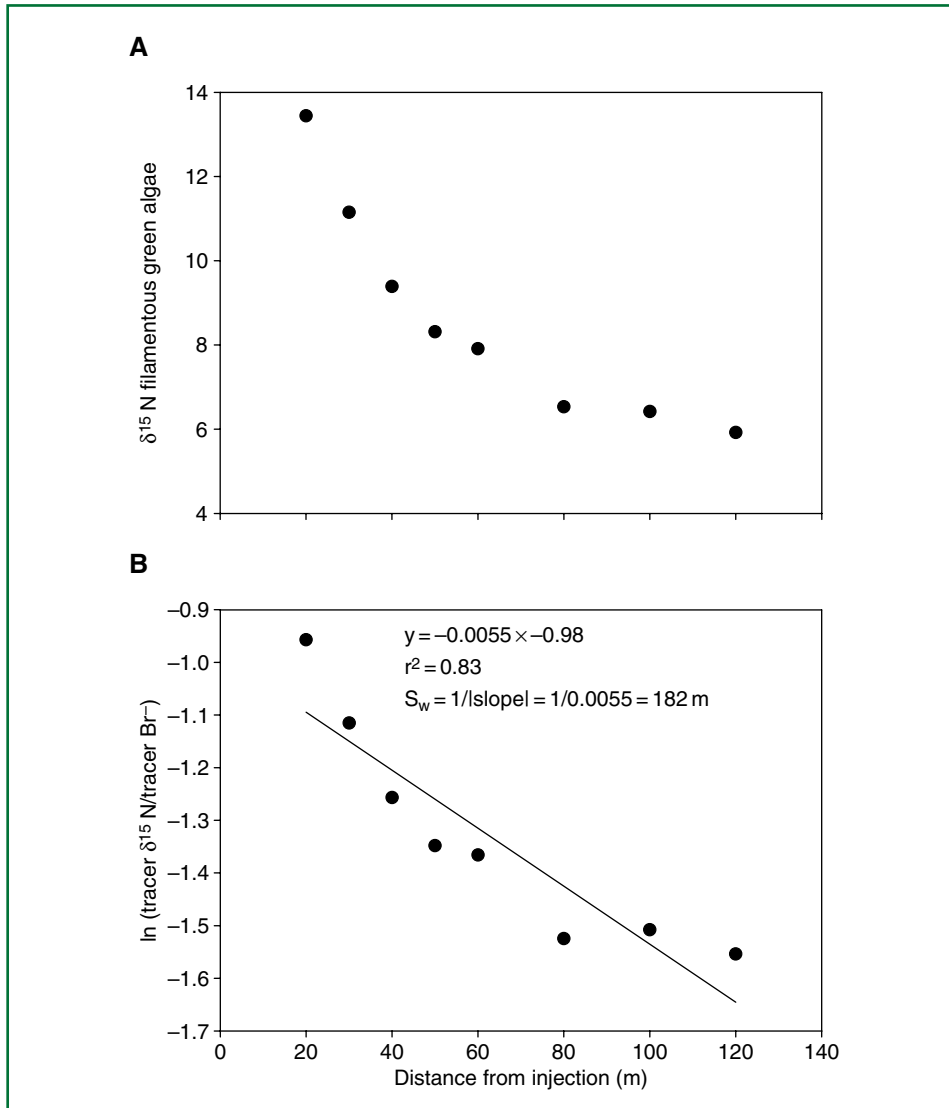
FIGURE 10.6 A. ^{15}N Enrichment (background-corrected) of filamentous green algae in a headwater stream in southwest Michigan after a short-term ^{15}N tracer release and B. same data plotted for calculation of uptake length, S_w, which is the inverse slope of ln(δ^{15}N:Br$^-$ concentration) over distance from the injection point.

V. MATERIALS AND SUPPLIES

Basic Method 1

Laboratory and Field Materials

> 2-L Erlenmeyer flask
> Stir/heat plates
> Granulated agar

Ammonium chloride (NH$_4$Cl)
Potassium phosphate (KH$_2$PO$_4$)
Sodium or potassium nitrate (NaNO$_3$ or KNO$_3$)
Ziploc®, snack size
L-shaped stakes
Parafilm
Cooler with ice
Polycon cups (1 oz; Madan Plastics #1's) with nutrient-diffusing substrate
Gray plastic L-bars (US Plastics #45031)
Fritted glass discs (i.e., glass crucible covers, Leco #528-042)
Small 4″ cable ties
Rubber silicon glue
Needle-nose pliers
Plastic wrap

Basic Method 2

Field Materials

Carboy
Bottle for filling carboy
Injection pump
Pump tubing with weight
Charged battery
Graduated cylinder
60-mL Water bottles
Disposable gloves
Ion specific probe
Field books
Weighed salts
Meter stick
Meter tape
Flow meter
60-mL Disposable syringes
Syringe filters
Cooler with ice
Stopwatch
flagging tape

Laboratory Materials

Ammonium chloride (NH$_4$Cl)
Potassium phosphate (KH$_2$PO$_4$)
Sodium or potassium nitrate (NaNO$_3$ or KNO$_3$)
Sodium chloride or bromide (NaCl or NaBr)
Disposable gloves

Advanced Method 1

Field Materials

Carboy
Bottle for filling carboy

    Injection pump
    Pump tubing with weight
    Charged battery
    Graduated cylinder
    60-mL Water bottles
    Whirlpak bags
    Disposable gloves
    Sieve
    Ion-specific probe
    Field books
    Weighed salts
    Meter stick
    Meter tape
    Flow meter
    Cooler with ice
    Stopwatch
    60-mL Disposable syringes
    Syringe filters
    Flagging tape

Laboratory Materials

    Coffee grinder
    Scintillation vials
    96-well plates
    Drying oven
    Isotope ($K^{15}NO_3$)
    Sodium chloride or Bromide (NaCl or NaBr)
    Disposable gloves
    4×6 mm tin capsules (Costech)
    Microbalance

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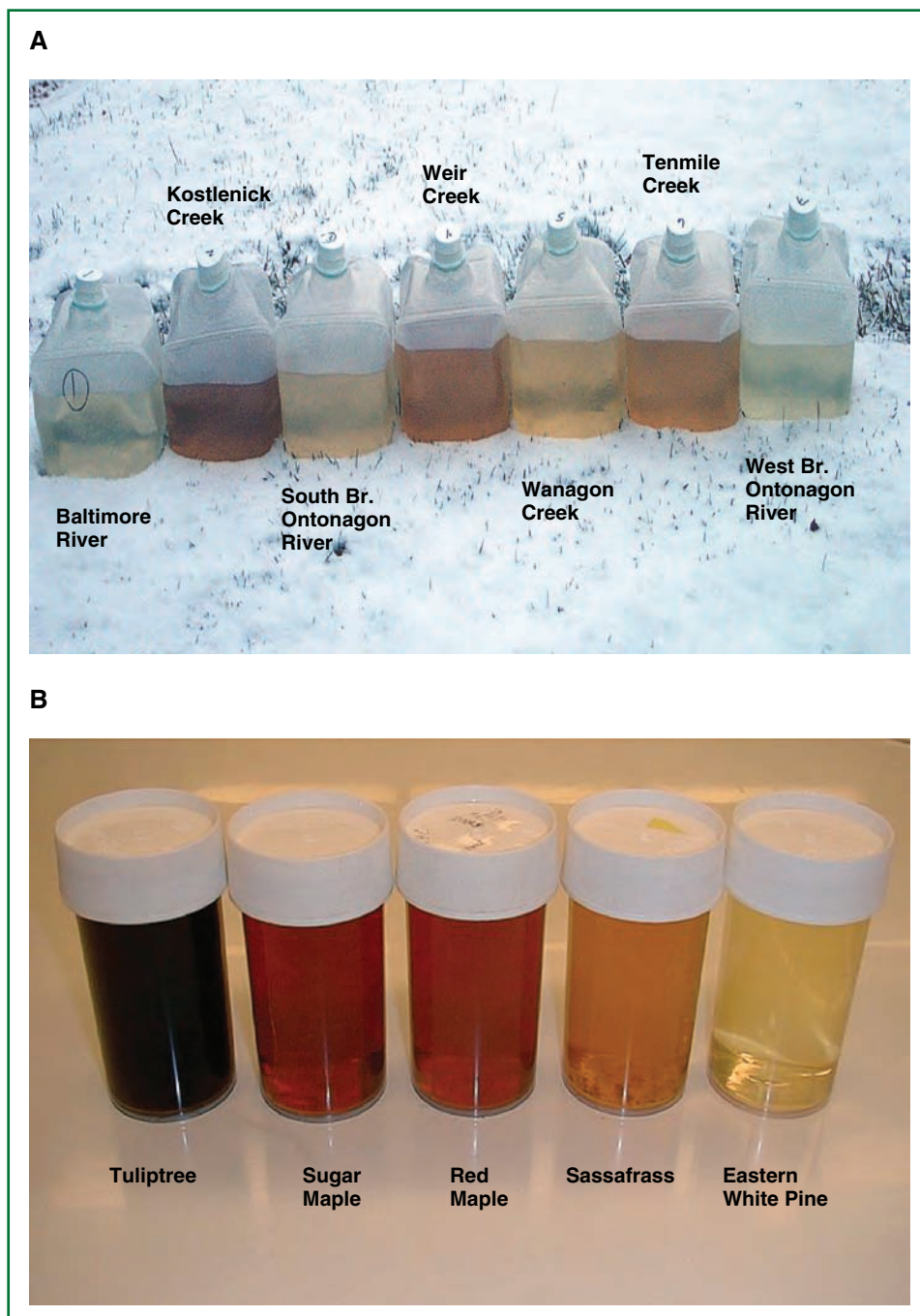
Dissolved Organic Matter

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I. INTRODUCTION

In stream ecosystems dissolved organic matter (DOM) is often a large proportion of total organic material standing stocks and is almost always the predominant component of downstream carbon flux (Webster and Meyer 1997). Typically, the concentration of DOM in headwater streams range from 1 to 5 mg/L of carbon, while streams draining high-organic soils or vegetated wetlands may have concentrations approaching 50 mg/L (Figure 11.1A). As an example, a stream with an average of 0.5 m water column and 2.5 mg/L carbon, translates into 2.5 g of carbon m^{-2}. Typically, this organic matter standing stock is small relative to particulate matter in debris dams but may be large relative to fine particles or algal biomass. In fact, the ratio of DOC to POC in transport is almost always >1 (Meybeck 1982). Indeed, the quantity of DOC passing a given point in a stream is typically severalfold greater than for POC, except under conditions of high flows carrying peak loads of suspended particles. As the flux of DOM is often the largest term in stream material budgets, one of the challenges facing stream ecologists has been detecting relatively small rates of DOM addition or removal given a large and typically variable background flux.

DOM has a composition similar to that of particulate organic materials including nitrogen and phosphorus in organic forms (amino acids, nucleotides etc.). However, for many analytical approaches only the carbon content is measured and so we refer interchangeably between DOM and DOC. From a conceptual view it is important to keep in mind that DOM includes a multitude of elements in addition to carbon. In particular, the organic forms of N and P are often released from organic material during degradation and are subsequently taken up by stream microbes (see Chapters 14 and 15). A complete analysis of the elemental and structural composition of DOM, down to the level of individual compounds, has never been completely described for any stream

FIGURE 11.1 Examples of dissolved organic matter in (A) streamwater samples from seven third- to fifth-order streams in the Upper Peninsula of Michigan ranging in DOC concentration from ~5–40 mg C/L (photo by J. Larson) and (B) leachates of fallen leaves from five different tree species found in the upper Midwest, USA. (Photo by E. Strauss.)

ecosystem although some generalizations are possible. The major DOM component in stream water is humic material; a broad class of organic acids containing aromatic rings (Thurman 1985). Identifiable monomers, such as simple sugars and amino acids, are readily detectable with current techniques (Kaplan and Newbold 2003) and although present in low concentrations may be particularly important in supporting heterotrophic activity. More complex carbohydrates, peptides, proteins, and nucleic acids are all present but in highly variable concentrations. Characterization of broad classes of compounds can be attained through measures of fluorescence and NMR (cf. Brown *et al.* 2004). The organic N and P contents of DOM also vary broadly (e.g., C:N ratios ranging from 10–50; Kaushal and Lewis 2003).

DOM derives from both within the stream (algae, macrophytes) and from external sources (soils, wetlands, fallen leaves). DOM is released by benthic algae and rooted macrophytes within streams and rivers. For instance, Kaplan and Bott (1989) were able to show diel fluctuations in bulk DOC in White Clay Creek implying that leakage during the photosynthetically active period was sufficient to alter concentrations. These autochthonous sources of DOC may be the more biologically available portion that occurs in lower concentration than the larger, arguably more stable pool of terrestrially derived DOM. Compounds released from living plants are generally of lower molecular weight and have fewer aromatic moieties than compounds leached from soils and litter (McKnight *et al.* 2001). Thus, DOM origin has a strong affect on decomposition rates and other aspects of DOC physicochemistry.

There have been many studies showing the importance of organic and/or poorly drained soils in a catchment contributing to stream DOC loads. For example, the annual flux of DOC can be predicted reasonably well by the quantity of wetlands in the catchment (e.g., Dillon and Molot 1997). Soil properties often contribute substantially to relationships between land-cover and stream DOM yield (Aitkenhead-Peterson *et al.* 2003). On smaller time and space scales, it is obvious that short duration hydrologic events (snow melt, storms) can lead to near-surface flow through high organic soil layers with a disproportionate influence on annual DOC export. For instance, it has been estimated that a large proportion of the annual yield of DOC from Rocky Mt. streams occurs during a brief period in the early spring (Brooks *et al.* 1999). Thus, DOM concentrations can vary dramatically over the span of a few hours and one of the difficulties in constructing mass balances is quantifying episodic export particularly when trying to balance these fluxes against *in situ* retention or metabolism.

Earlier views on the bioavailability of DOM tended toward the opinion that many of the compounds comprising DOM were refractory to biotic degradation in a sense representing the "leftovers" from decomposition in terrestrial systems (cf. Kaplan *et al.* 1980). More recently it has become clear that DOM is consumed/metabolized within streams and rivers given a sufficient opportunity to interact with benthic and hyporheic biofilms (see Chapters 6 and 33). DOM may also be adsorbed by mineral surfaces and biofilms and retained in a stream reach for subsequent metabolism (Freeman and Lock 1995). Sorption of DOM to biofilms may well be the first step in eventual metabolism and can serve as a scavenging and buffering mechanism enhancing retention of DOM in a stream reach. Deposition on mineral surfaces is particularly important during movement of DOM through soil profiles, certain minerals have a great potential for removal and protection of DOM (McCracken *et al.* 2002) leading to changes in concentration and composition.

Aside from biotic assimilation within sediments, DOM is also susceptible to direct photomineralization and alteration in bioavailability following exposure to full sunlight

(Weigener and Seitzinger 2001). The energy in solar radiation interacts particularly strong with certain classes of compounds (e.g., complex phenolics) resulting in direct release of CO_2, small organic molecules, and often a decrease in light-absorbing capability (i.e., photobleaching) (Moran and Zepp 1997). These chemical transformations are often, but not always, accompanied by an increase in the ability of a DOM mixture to support bacterial growth, presumably due to "priming" of complex compounds for biotic metabolism or release of specific, directly assimilable compounds (Tranvik and Bertilsson 1999). Photolytic effects on DOM in streams and rivers are diverse with some reports of strong positive effects of light-exposure on DOM degradation with others showing neutral or negative effects (e.g., Findlay *et al.* 2001, Wiegner and Seitzinger 2001, Brisco and Ziegler 2004).

From a more anthropocentric point of view, DOM abundance and characteristics affect both contaminant transport and drinking water quality. DOM interacts with organic contaminants acting as binding sites for some less-soluble organic compounds or interaction with metals (e.g., McKnight *et al.* 1992, Chin 2003). Methyl mercury concentrations in an array of surface waters were positively correlated with bulk DOC (Dennis *et al.* 2005). The quantity of DOM is also relevant for drinking water supplies since excess chlorination of high DOM waters can result in the formation of halogenated compounds with potentially carcinogenic properties.

II. GENERAL DESIGN

As a functional definition, DOM ranges from truly dissolved to almost colloidal material. As a consequence, the choice of filters used to capture particulates and produce a DOM filtrate has undergone some convergence and agreement toward accepted uniformity, but is ultimately arbitrary. In general, water samples are filtered through glass-fiber filters having a nominal pore size of $\approx 0.5 \mu m$, although nylon and nitrocellulose and other esters can also be used following pre-rinsing with sample. Samples can be preserved with refrigeration over the short term (days). For longer term storage, samples can be kept frozen, although freezing can lead to irreversible precipitation of less soluble compounds. Alternatively, a variety of preservatives (e.g., acidification, mercuric chloride) can be used for longer term storage (Kaplan 1994), but they may interfere with subsequent analyses for specific compounds.

The actual quantification of DOM is usually focused on the carbon compounds. The primary method of analyzing a sample for carbon content is combustion with catalytic oxidation to CO_2 followed by detection with an infrared gas analyzer (Sharp *et al.* 2002). There are numerous instruments available for carbon analysis, and most are automated. The N and P components can be analyzed as the difference between total dissolved nitrogen (TDN) or total dissolved phosphorus (TDP) and the inorganic forms (Chapters 8–10). However, it is generally preferable to measure DON and DOP directly given the compounding of error in the estimation of concentration by difference.

The composition of DOM is very complicated and a complete chemical characterization of its organic constituents generally is not possible. As for bulk DOM, many of the constituents are defined functionally, for instance fulvic and humic acids are separated based on solubility at low pH (Thurman 1985). Several selective resins targeted at the humic acid fraction have been used for both characterization and preparative isolation. Given the analytical complexity, a host of coarser-resolution characterization schemes

have been proposed and shown to offer ecologically relevant information about DOM sources and dynamics. Gross molecular weight (MW) distribution can be determined with a series of nominal MW cut-off filters (Leff and Meyer 1991) and there is a wide array of spectrophotometric and fluorometric bulk assays. These are built upon the absorbance and fluorescent characteristics of certain compounds and functional groups generally associated with the phenolic sub-units of high molecular weight organic acids. For example, the carbon-specific fluorescence index (McKnight *et al*. 2001) has been used as an indicator of the relative contribution of aromatic versus aliphatic compounds in the DOM mixture.

III. SPECIFIC METHODS

The following methods are intended to demonstrate multiple sources of DOM and the removal of DOM from water by heterotrophs, photolysis, and sorption in soil.

A. Basic Method 1: Multiple Sources of DOM

Potential sources of DOM to a water body include inflowing surface waters, throughfall, porewater in local wetlands or high organic soils, and groundwater (see Chapter 31). Depending on the system under consideration, these samples are easy to collect although the timing may be critical for episodic inputs. Sampling sites and timing should be established based on research questions about DOM dynamics of interest before embarking on a sampling program, such as the one described next.

1. Collect water samples in 125-mL acid-washed and DI-rinsed bottles. Amber-colored bottles are recommended to reduce photodegradation of the DOM.
2. Filter water either in the field or immediately upon return to the laboratory using a precombusted 0.5-μm glass fiber filter. Samples can be held in the refrigerator or acidified (pH 2) for longer term stability.
3. Determine concentrations with an automated TOC analyzer (e.g., Shimadzu 5000) or in some cases by measuring the absorbance in a 5-cm spectrophotometer cell at 400 nm.
4. Obviously, one needs to know the concentration of DOM per unit volume and the actual quantity of water entering via the different pathways to arrive at a useful comparison of various potential sources.
5. To augment field sampling, various potential sources can be examined with lab-based leaching experiments. For example, different leaf types (separated by species or apparent degree of decay) can be leached under standard conditions to determine their potential contribution to surface water DOM (Figure 11.1B). Similarly, different soil types representing a range in organic contents can be subjected to a standard leaching treatment to determine their relative potential as a source of DOM.

B. Advanced Method 1: Heterotrophic Activity of DOM

If direct analysis of DOC is available, a range of experiments can be used to reveal differences in heterotrophic DOC removal under either field or laboratory conditions.

The simplest approach is to collect water samples from various sources/conditions such as (1) streams before and just after leaf fall, (2) riparian wetland versus open channel, or (3) groundwater seeps versus open channel.

1. Isolate water in 500-mL bottles or flasks and follow concentration of DOC over time.
2. Loss from the more labile sources should be observable within two weeks and is detected as a decline in concentration or absorbance.
3. Simple dissolved oxygen measurements (see Chapter 5) can be used to confirm differences in metabolism across treatments to account for the possibility that different rates of DOC loss were simply due to precipitation/sorption.
4. Depending on source water nutrient content, it may be informative to add a nutrient amendment treatment to assess whether N or P limitation might be influencing DOM degradation.
5. If feasible, particularly in conjunction with hyporheic water sampling (see Chapter 6), a set of water samples from different locations along hyporheic flowpaths can be used to track changes in DOC and dissolved oxygen.
6. Determine net loss of bulk DOC by measurement of concentration following procedures above as appropriate for the available instrumentation. If absorbance is used, it must be kept in mind that absorbance may change due to shifts in relative abundance of chromogenic compounds without a concomitant decline in bulk DOC concentration. Parallel samples to follow biological oxygen demand (see Chapters 5 and 14) may help resolve this question.

C. Advanced Method 2: Enzymatic Characterization of DOM

Quantifying the components of DOM actually supporting heterotrophic metabolism remains one of the most complex challenges in the field. With the diversity of compounds (many highly complex and poorly characterized) and the diversity of microbes, the possible interactions and variability are daunting.

1. We have used a series of extracellular enzyme assays to describe differences in DOM components undergoing attack (cf. Findlay *et al.* 2001). The presumption is that changes in the units of DOM being degraded are accompanied by shifts in allocation among enzymes targeting different classes of compounds.
2. If proteins become relatively more important as a growth substrate for stream bacteria, one would predict increased peptidase activity. Interpretation of these enzymes degrading organic carbon compounds differs from enzymes targeting specific elements that may be available from either inorganic or organic sources. For instance, high phosphatase activity is often viewed as indicative of P limitation since the cells have essentially "switched" to an organic form of P. For the organic carbon degrading enzymes the analogy would be that absence of monomers triggers degradation of the polymeric form.
3. One important difference is that end products of polymer hydrolysis may be used for catabolism rather than as building blocks and so even if the monomer is present there is still some benefit from polymer degradation. Therefore, high abundances of polymers are generally expected to induce the appropriate enzyme.
4. Enzyme activities can be measured with either spectrophotometric or fluorometric approaches; the latter requires smaller volumes of sample and substrates but does

require a plate-reading fluorometer. The spectrophotometric methods use PNP-linked substrates and PNP is released as the model substrates are cleaved. Bacterial films growing in the dark on inorganic substrates (buried stones, shaded ceramic tiles) are a useful model community since they rely on supply of organic carbon from the water column. For the PNP substrates it is possible to incubate actual stones in solutions of streamwater and substrate although this generates a fair amount of waste and requires larger quantities of the substrates. The procedure can be scaled down by scrubbing the biofilm off the stones/tiles and resuspending in streamwater or buffer to which small quantities of substrate are added. If a plate reader is available multiple assays are possible on a single plate greatly expediting sample processing. Also the plate can be read repeatedly allowing better resolution of the time course of color or fluorescence development.

5. Some enzymes (esterases, proteases) consistently develop color (or fluorescence) faster than some of the carbohydrases. If biofilm suspensions are used, these are released by vortexing or scrubbing from several cm^2 of surface and resuspended in ~5 mL of streamwater. These sample suspensions (150 μL) are mixed in the plate wells with 100 μL of substrates made at a concentration of 1 mM (Table 11.1). Blanks consisting of substrate with no biofilm suspension, biofilm with no substrate or even autoclaved biofilm suspension are run in parallel to detect any instability in substrates.

6. Complete the following protocol:
   a. *Bicarbonate buffer*: Make a 100 mM stock solution by dissolving 8.4 g $NaHCO_3$ in 1.0 L of deionized water. Stock solution pH is 8.2. Dilute 50 mL of stock solution to 1.0 L to make 5 mM solution for substrates. Note that some substrates (Leu-aminopeptidase, esterase) are unstable in buffer and should be made up in autoclaved deionized water.
   b. *Example microplate setup*: Each microplate has 8 rows and 12 columns.
      Column 1: reference blank, 250 μL 5 mM bicarb buffer solution
      Column 2: background, 200 μL fresh water sample + 50 μL bicarb buffer
      Columns 3–12: assays, 200 μL fresh water sample + 50 μL of substrate solution.
   c. Dispense water sample and blanks first, and substrate solutions last.

TABLE 11.1	Preparation of 1000 μM Solutions of Methylumbelliferyl (MUB) Linked Substrate in 5 mM Bicarbonate Buffer.
Enzyme	**Substrate**
Esterase	4-MUB-acetate
	4-MUB-propionate
	4-MUB-butyrate
Phosphatase	4-MUB-phosphate
Leu-aminopeptidase	L-Leucine 7-amido-4-methyl-coumarin
β-glucosidase	4-MUB-β-D-glucoside
α-glucosidase	4-MUB-α-D-glucoside
β-xylosidase	4-MUB-β-D-xyloside
β-N-acetylglucosaminidase	4-MUB-N-acetyl-β-glucosaminide
endopeptidase	4-MUB-P-guanadinobenzoate

d. Warm up plate reader for 1 hr. Set excitation wavelength at 365 nm and emission wavelength at 450 nm. Place the plate into the microfluorometer, read immediately, and read again at 0.1–1.0 hr intervals, depending on activity.

e. After confirming that reaction rates were linear over the period of assay, calculate activity as nmol substrate converted per hour per mL of water sample:

$$Act = \frac{(FU_{mf} - FU_{mi})}{4100 \times 0.4 \times t_h} \qquad (11.1)$$

where Act is expressed in nmol h^{-1} mL^{-1}, 4100 is the nmolar emission coefficient (at pH 8), 0.4 is the water sample size (mL), and t_h is assay time interval in hours.

f. If biofilm suspensions have differing degrees of turbidity, color, or pH, an internal spike with known quantity of MUF can be used instead of assuming a constant efficiency of fluorescence detection across all samples. Data are typically analyzed with multivariate techniques (e.g., Principal Components Analysis) to reduce the multiple enzyme assays to two or three dimensions.

D. Advanced Method 3: Photolysis/Photobleaching of DOM

This method can be conducted even if direct DOC analysis is not available. Some DOC samples will show obvious declines in absorbance, which may even be detectable with the naked eye. Often a simple spectrophotometric absorbance measurement will show changes. These optical properties do not guarantee that there has actually been a change in total mass of DOM but certainly suggest changes in composition. These measurements can be coupled with measures of oxygen consumption in sealed bottles of treated water to determine whether the degradability of DOM has been altered by exposure to sunlight.

Experimental manipulations should include exposure to full sun in an open pan or quartz glass bottle, selective filtering with UV-blocking materials (e.g., Mylar) and, of course, a dark control. Ideally the exposure is related to real-world conditions. For instance, one might estimate the length of time that a water mass requires to travel through an open canopy reach of stream (see Chapters 8 and 10). As above, the required measurements are simply the change in DOM over time estimated via DOC quantification or change in absorbance.

E. Advanced Method 3: Sorption of DOM in Soils

Different soil types and horizons can variably act as sources or sinks for DOC moving in soil water. Small columns of organic (with or without leaf litter) or mineral soil can be perfused with solutions of DOC (or distilled water) to determine whether they are net contributors or sinks for DOC. As for the photolysis experiment, it may be possible to detect changes in DOC by simple spectrophotometric measurements if an analyzer is not available. It is also feasible to use the effluents from these columns as source waters for the bioavailability/metabolism assays described above.

IV. QUESTIONS

1. Based on DOC concentrations and volumes of water entering a stream reach (or other water body), what are the largest sources of DOC? Are the sources with highest concentrations the largest overall contributor?
2. What DOC sources are most variable over various time scales?
3. What litter types (or ages) have greatest potential to contribute DOC?
4. What DOC sources are most susceptible to heterotrophic metabolism? What sources are most susceptible to photolysis?
5. If a change in absorbance occurs after exposure to sunlight how large an effect will this have on light penetration? Are there differences in enzyme "fingerprints" among sources, streams? Which enzymes are most sensitive?
6. What soil types or layers show greatest capacity to supply versus remove DOC from solution? Is DOM from various sources equally susceptible to sorption to mineral soils?
7. After DOC passes through sorptive soil layers can it support heterotrophic activity? Are soils in your region likely to be net sources or sinks for DOM?

V. MATERIALS AND SUPPLIES

$0.5 \mu m$ glass fiber filters
Filtration apparatus
$NaHCO_3$
Deionized water
Microplate
5 mM Bicarbonate buffer solution
Leu-aminopeptidase substrate
Esterase substrate
Microfluorometer
Assorted laboratory glassware

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Transport and Storage of FPOM

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I. INTRODUCTION

Fine particulate organic matter (FPOM) includes particles in the size range of $>0.45\,\mu m$ to $<1000\,\mu m$ (1.0 mm) that are either suspended in the water column or deposited within lotic habitats. Size fractions of FPOM can be further divided into the categories of medium-large ($250–1000\,\mu m$), small ($100–250\,\mu m$), fine ($45–100\,\mu m$), very fine ($25–45\,\mu m$), and ultrafine ($0.45–25\,\mu m$). Suspended fine particulate material is referred to as *seston* and includes all living (e.g., bacteria, algae, protozoans, invertebrates, etc.) and nonliving material (amorphous organic matter, detritus, as well as suspended inorganic sediment) within the 0.45-μm to 1-mm size range. Seston can originate from many sources, including the breakdown of larger particles by physical forces, animal consumption, microbial processes, flocculation of dissolved substances, and terrestrial inputs (Wotton 1984, 1990). Transported loads of seston vary greatly among lotic systems from micrograms in some small streams to metric tons in larger streams and rivers (see Chapter 7). Seston functions as an important food resource for many filter-feeding invertebrates (Wallace and Merritt 1980, Benke *et al.* 1984), as well as for some vertebrates, such as paddlefish (*Polyodon spathula*), in large rivers. In some situations, such as below the outflow of dams or lake outlets, filter-feeding populations can remove large portions of transported seston from the water column within a few kilometers (Maciolek and Tunzi 1968, Voshell and Parker 1985). Dense populations of filter-feeding black flies

can also transform large quantities of dissolved organic matter and FPOM in rivers into much larger fecal pellets (Wotton *et al.* 1998, Malmqvist *et al.* 2001), which increases the rate of particle deposition. The downstream transport of seston is also important to the theme of conceptualizing streams as longitudinally linked systems (Vannote *et al.* 1980, Minshall *et al.* 1985) and the concept of material spiralling in stream ecosystems (Newbold *et al.* 1982). Therefore, seston is important to many ecosystem processes as it represents a major pathway of organic matter transport and export and is thus an important consideration in ecosystem organic matter budgets (e.g., Fisher and Likens 1973, Cummins *et al.* 1983, Golladay 1997, Webster and Meyer 1997).

FPOM occurs not only in the water column as seston but is also found deposited in lotic habitats as fine benthic organic matter (FBOM). FBOM standing crops are rarely adequately assessed in stream research. Sometimes FBOM is ignored completely, or measurements are done in conjunction with benthic sampling for macroinvertebrates with a relatively large mesh size (e.g., 250 μm) that underestimates the total stored FBOM. For example, Minshall *et al.* (1982) found that standing crops of benthic organic matter may be underestimated by as much as 65% when sampling devices with 250 μm meshes are used. Additionally, standing crops of organic matter may vary greatly between erosional (e.g., riffles and outcrops) and depositional areas (e.g., pools) of streams. Debris dams, for example, often are sites of high FBOM storage (Bilby 1980, Smock *et al.* 1989). FBOM and associated microbes serve as an important resource for animals adapted for deposit feeding (collector-gatherers; see Chapter 25), which includes a wide assortment of invertebrates as well as some collector-gathering fishes ("rough" fishes). Many deposit-feeding animals have low assimilation efficiencies, and the ingestion and reingestion of FBOM and associated microbes may occur many times in longitudinally linked systems. Unfortunately, only a few studies have attempted to quantify the turnover of FPOM; Fisher and Gray (1983) estimated that fine particle feeders ingested over four times their weight per day, and the entire standing crop of FPOM in Sycamore Creek, Arizona, was ingested and egested every two to three days. FBOM storage varies greatly within heterogeneous stream environments. In small headwater streams, the highest standing crops of FBOM are usually associated with pools and wood debris dams (Bilby and Likens 1980, Huryn and Wallace 1987, Smock *et al.* 1989). In large river systems, slackwater habitats such as sloughs and backwaters are repositories for large amounts of FBOM; during high flow conditions floodplains adjacent to large rivers can serve as both source and sink of seston and FBOM (Grubaugh and Anderson 1989).

A number of approaches have been used to estimate FPOM quality and will only be mentioned here. Organic:inorganic matter ratio is simply an estimate of the relative amount of organic and inorganic matter in seston and can be easily determined from procedures described below for seston sampling (see Option 1). This ratio often varies greatly for different size fractions of seston, with smaller size classes having a greater proportion of inorganic material (ash) than larger size fractions. Some studies (e.g., Angradi 1993a) have examined the organic constituents of seston such as chlorophyll *a* (see Chapter 17), while others have examined other organic material and microbial activity such as respiration (Peters *et al.* 1989, Bonin *et al.* 2000, 2003; also see Chapter 28 for examples of respirometry techniques). Edwards (1987) evaluated the importance of bacteria in seston and in the growth of filter-feeding black fly larvae (Edwards and Meyer 1987). Carlough and Meyer (1991) found sestonic protozoans to be an important component of seston in a low-gradient, blackwater river. Voshell and Parker (1985) used microscopy to examine directly the frequency and type of particles in various size categories. The amounts in each category (e.g., animal, diatoms, other algae, vascular

plant, and amorphous detritus) are estimated by the areal standard-unit method used in phytoplankton studies as described by Welch (1948). Wallace *et al.* (1987) used a microscope and digitizer interfaced with a computer for similar analyses; however, these latter methods are not appropriate for bacteria and protozoans.

More recently, other "higher-tech" methods have been employed to study dynamics and origin of seston FPOM. For example, radioactively tagged particles have been used to study movement and deposition of seston (Cushing *et al.* 1993, Minshall *et al.* 2000, Monaghan *et al.* 2001, Thomas *et al.* 2001). Hall *et al.* (1996) estimated transport distances of bacteria by tagging them with fluorescent markers. Several seston analogs have also been used to examine rates of transport and deposition including corn pollen and glass beads (Miller and Georgian 1992, Webster *et al.* 1999, Georgian *et al.* 2003), *Lycopodium* spores (Wanner and Pusch 2000), and yeast (Paul and Hall 2002). Angradi (1993b) used stable carbon and nitrogen isotope analysis to study origin and movement of seston. Raymond and Bauer (2001) used ^{14}C to find that several rivers transport very old (often >1000 yr) seston originating from terrestrial soils.

In the following exercises, we will describe seston sampling procedures for streams and rivers of various sizes and describe techniques to assess seston concentration, size distribution, and instantaneous estimates of total seston export. Next we will consider sampling techniques for FBOM in streams and emphasize the relative importance of depositional and erosional habitats in assessment of FBOM standing crops. The final exercise examines direct linkages between sestonic FPOM and filter-feeding biota. The specific objectives of these exercises are to (1) introduce the reader to the importance and magnitude of FPOM transport in streams; (2) demonstrate techniques for collecting and analyzing seston and FBOM; (3) demonstrate the importance of hydrologic events in seston transport compared to base flow conditions; (4) compare the relative importance of erosional and depositional habitats in FBOM storage in streams; and (5) illustrate direct consumption of suspended particles by filter-feeding larvae of black flies (Diptera: Simuliidae). The reader should gain an appreciation for the methods involved in assessing FPOM transport, storage, and use in streams.

II. GENERAL DESIGN

A. Seston

Instantaneous seston concentrations (e.g., mg/L) can be easily measured by filtering known volumes of water through preashed and preweighed glass-fiber filters. This simple approach can be used to compare seston concentrations during base flow and short-term hydrologic events (e.g., storms), or for comparing seston concentrations among streams of various sizes. Percent ash in such measurements has been related to long-term watershed disturbance (Webster and Golladay 1984). In some cases, seston particle size has been shown to vary with stream size, as smaller headwater streams draining forested areas have larger median particle sizes than larger rivers downstream (Wallace *et al.* 1982). However, with few exceptions, the majority of the particles transported by most streams during baseflow conditions are <50 µm in diameter (Sedell *et al.* 1978, Naiman and Sedell, 1979a, 1979b, Wallace *et al.* 1982).

A two-part sampling approach generally is adequate for sampling seston in small rivers and streams. The first part consists of collecting a 20 to 30-L grab sample for measuring concentrations of finer seston particles (i.e., <250 mm). Under most conditions, seston particle-size distributions are strongly skewed toward smaller size fractions; therefore, the
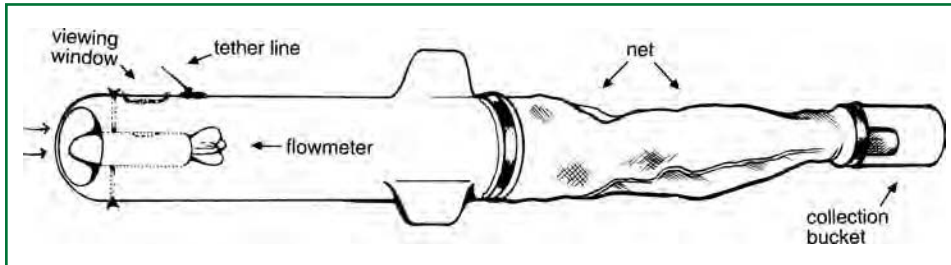
FIGURE 12.1 A Miller-type tow net, equipped with a flowmeter to record velocity of water filtered. The flow meter is used to calculate distance of water filtered over the time interval the net is deployed (see seston concentration protocols and Table 12.1). A Plexiglas® viewing window (optional) is ideal for viewing the dial on the current meter.

second part of sampling uses a collection net to filter a large quantity of water to obtain reasonable concentration estimates for larger seston particle sizes (i.e., $>250\,\mu m$). One of the best devices for this purpose is a Miller plankton tow net fitted with a 250-μm mesh collecting net and flowmeter (Figure 12.1).

Particle size separation requires a wet filtration system consisting of a series of stacked sieves of Nitex® or bolting-cloth netting of various sizes. Sieves can be constructed with short (4–5 cm) sections of PVC pipe with netting glued over one end and joined with connectors to form a stackable series of sieves. More elaborate wet filtration systems are constructed of threaded, stainless steel tubes fitted with stainless steel bolting-cloth filters of various dimensions. The individual filters with Teflon® gaskets are inserted between threaded sections of each tube to form a series of stackable sieves with a large funnel at the top of the apparatus (Figure 12.2). A water sample is poured into the funnel and through the sieves under vacuum. Seston particle sizes are thus separated by sieve sizes and water passing though the smallest filter can be retained for the ultrafine fraction.

Large rivers ($>$7th order) present considerable difficulties for seston sampling. Most of these rivers are nonwadeable and sampling can only be conducted from bridges or boats. Furthermore, as a result of differential settling rates and lower current velocities near the water/substratum interface, seston concentrations and particle-size distributions can vary greatly with depth as well as with sampling location relative to the *thalweg*, or middle of the main channel. Adequate sampling of suspended material in large rivers requires depth-integrated, velocity-weighted samples taken at multiple depths along a transect. A variety of devices have been designed to collect integrated and weighted samples of total suspended sediments and these lend themselves well to seston sampling in large rivers (e.g., Grubaugh and Anderson 1989; see also Chapter 7). Drawbacks to their general use are that these samplers are expensive and require a trained operator. The reader should consult Guy and Norman (1970) for a discussion of such devices and sampling designs for use in large rivers.

Point samplers, although considerably less accurate than depth-integrating devices, are much less expensive, easier to operate, and can also be used in large rivers. The protocol provided in this exercise for large river seston collection employs point-sampling techniques. Examples of point samplers include Kemmerer or Van Dorn bottles fitted with *line depressors* or fluked weights to facilitate a vertical descent of the sampler in high-velocity rivers. To estimate seston concentration using point samplers, samples need to be collected at several locations along a given vertical gradient, with the depth and number of samples dependent on the total depth of the water column.
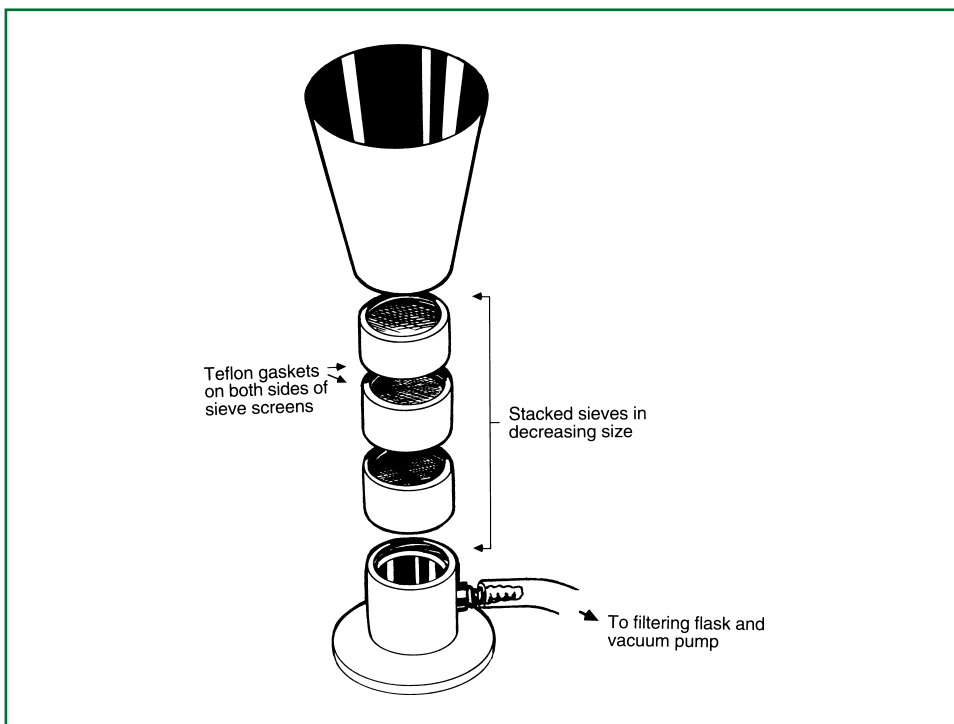
FIGURE 12.2 Example of a wet filtration system using a series of stacked sieves in order of decreasing size. *Note*: Vacuum line is connected to a large filtering flask to retain filtrate for ultrafine seston and to avoid pumping water into the vacuum pump (see particle size separation protocols).

Traditional methods of FPOM collection (see above) are limited in their ability to capture the spatial variability of FPOM in large rivers. Newer methods relying on remote sensing techniques are likely to become an important tool for quantifying FPOM load in large rivers. These techniques take advantage of the colors associated with suspended matter. For example, turbidity in the Great Miami River, Ohio was related to specific wavelengths measured by an airborne hyperspectral sensor (Shafique *et al.* 2001). Using this relationship the authors produced maps displaying the relative distribution of turbidity for 80 km of river. These maps showed plumes of clear-water (i.e., low turbidity) associated with a wastewater treatment plant. Similar relationships with total suspended solids (TSS) have been found in other freshwater systems (e.g., Oron and Gitelson 1996, Dekker *et al.* 2001). Although turbidity and TSS describe both inorganic and organic particulate matter, FPOM alone can be calculated using the proportion of organic matter estimated from traditional sampling (Dekker *et al.* 2001). Additional applications of remote sensing techniques in lotic systems are discussed by Johnson and Gage (1997) and Mertes (2002).

B. Fine Benthic Organic Matter

Laboratory protocols similar to those employed for seston can be used to determine stored FBOM concentrations and particle size distributions, if samples are resuspended in a known volume of water prior to analysis. Sampling procedures, however, can be

more complicated for FBOM storage, as stream characteristics such as current velocity, substrate particle size, and the presence or absence of retention devices must also be taken into consideration. These factors influence both the physical storage of FBOM as well as the structure of the benthic community (e.g., Huryn and Wallace 1987) and hence affect transport and use of FPOM in lotic systems. To assess these influences, it is necessary to measure standing stocks of FBOM among stream habitats with differing morphological characteristics. For the purposes of this chapter, FBOM will be quantified in three size fractions: 0.45 to 250 μm, 250 to 500 μm, and 500 μm to 1.0 mm. Particle sizes that are greater than 1.0 mm constitute coarse particulate organic matter (CPOM) and are addressed in Chapter 13.

C. Linkages

Collector-gatherer and collector-filterer organisms use FBOM and seston, respectively, as food resources (see Chapter 25). One collector organism is the larval black fly, which uses cephalic fans to filter and remove small suspended particles (<300 μm diameter) from the water column. Black flies and other collector-filterers are important in energy transformations in streams and are examples of direct linkages between seston and the biota (Wallace and Webster 1996). Larval black flies also make good study organisms since they are often very abundant in many lotic habitats and have rapid (<1 hr) gut passage times (e.g., Wotton 1978, 1980).

We will expose larval black flies to a dense concentration of trackable FPOM (powdered charcoal) for a brief time, which will produce a distinct band in the larval guts. Representative larvae (preferably of different instars or sizes) will be collected and preserved at 10, 20, and 30 minute intervals following particle release. In the laboratory, larvae from each time interval will be separated by size class (body length) and their guts dissected. Using a dissecting microscope equipped with an ocular micrometer, we will make two measurements: the distance from the posterior end of the head to the band of charcoal, and the distance from the posterior end of the head to the tip of the abdomen. The ratio of these two (ratio W; Wotton 1978) gives a measure of gut passage time based on the distance the band has moved.

D. Site Selection

1. Seston

Methods described herein are designed for both small streams and rivers; however, if used for class purposes we recommend that they be restricted to lotic reaches that are safely wadeable. Application of this protocol to larger rivers requires working from either bridges or boats, both of which carry inherent risks. If a bridge site is used, the bridge should be close enough to the water surface, and water depth should be shallow enough that sampling equipment can reach the river bottom. The bridge should have sufficiently wide shoulders and limited automotive traffic to facilitate safety; sampling crews are strongly urged to exercise extreme caution and to wear "blaze-orange" garments to enhance their visibility. Boat sampling requires that attention be given not only to sampling equipment and procedures but also to boat maintenance, proper safety equipment, and safe boating practices. Because of inherent dangers of sampling during

high-flow conditions, we recommend boat sampling be conducted only at low- to mean-flow stage conditions. Seston sampling during storm events can be dangerous even in small streams and adequate precaution is again recommended.

Management of the sampling site is as important as site selection; disturbance of upstream substratum should be avoided when collecting water for seston analysis. Even minor disturbances such as wading across a stream can dislodge sufficient amounts of FPOM to greatly increase seston concentrations well above baseflow conditions. Be especially careful when working with a field team consisting of several individuals; the person or persons actually collecting seston samples should be upstream of other team members. Finally, the volume of water filtered or collected with the seston is crucial information in seston sampling. Care should be taken in both the field and laboratory when determining and recording volume measurements.

Frequently, especially when assessing the effects of ecosystem-level disturbances, it is desirable to know how FPOM transport responds to individual storms for disturbed and reference conditions because many studies have shown much greater concentrations and transport of seston during storm discharges. Hewlett and Hibbert (1967) have provided a method for determining what constitutes a storm for catchments of various sizes. Many investigators have noted that the concentration of particulates increases rapidly during the rising limb of the hydrograph, and that peak concentrations are somewhat unpredictable, usually occurring before peak discharge (Fisher and Likens 1973, Bilby and Likens 1979, Gurtz *et al.* 1980, Fisher and Grimm 1985, Webster *et al.* 1990, Wallace *et al.* 1991). Automated sampling device such as those manufactured by ISCO (ISCO, Inc., Lincoln, Nebraska) can be used to obtain samples of up to 1 L at various intervals during individual storms. Storm sampling, including a sequence of samples taken over rising and falling hydrographs, can be initiated by wetness (rainfall) sensors of individual storms, or sufficient rise in stream hydrographs (Golladay *et al.* 1987, Wallace *et al.* 1991, Harmel *et al.* 2003). ISCO samplers, as well as several other commercially available devices, offer somewhat similar options such as programmable operation and memory, water-level or stage recorders, water-level collection devices, including sample collection pump and storage, and discrete versus composite samples. Some negative aspects of such devices include high initial costs and high maintenance requirements. Various automated collection devices, settings, and sampling strategy considerations, etc. are discussed by Harmel *et al.* (2003).

2. Fine Benthic Organic Matter

In this method, logistical considerations are again of primary concern. The protocols for this method can most easily and safely be accomplished in relatively shallow streams (<0.75 m depth) that are readily wadeable. Furthermore, site selection should be focused toward stream reaches with clearly heterogeneous channel features consisting of zones of contrasting current velocities, such as pools and other clearly depositional reaches versus cobble riffles or bedrock outcrops. If the site selected is in a nonwadeable, medium-sized stream or river that is too deep for devices described in the FBOM Method, SCUBA gear in combination with elaborate sampling devices such as dome samplers can be used to sample benthos and FBOM (Gale and Thompson 1975, Platts *et al.* 1983). Such devices are expensive and will require trained operators. In backwaters or other slow current habitats, an Eckman dredge can be used, but this device often has a high loss of FBOM. In habitats with slow to moderate currents (e.g., channel borders and sloughs) a Ponar or petite Ponar dredge can be used; in moderate to fast currents (e.g., main channel)

Peterson dredges are preferred. None of these dredges will function properly if the site selected has coarse substrata.

3. Linkages

This method is most easily performed in small streams less than 0.25 m in depth. The site should have a reasonably abundant black fly population; shallow outflow streams from lakes and small reservoirs are ideal locations because black fly larvae often form dense aggregations at such sites. It is also important to select areas where the charcoal slurry can be released immediately upstream of the black flies so that the slurry plume flows directly over the larvae.

Sampling site management is again important; use caution to minimize disturbing black fly aggregations prior to, during, and following release of the charcoal slurry. When collecting larvae following charcoal release, stand to one side of the area covered by the charcoal plume and remove larvae by carefully reaching into the area covered by the plume.

III. SPECIFIC METHODS

A. Basic Method 1: Seston Concentration

1. Protocol for Seston Sampling in Streams and Small Rivers

1. For obtaining a *carboy* sample (steps 1–3): cover the opening of a clean, 20 to 30-L carboy with a 250-μm mesh sieve or bolting cloth.
2. Using another carboy, bucket, or other vessel, collect a grab sample of stream water. Care should be taken not to disturb the substratum and collect resuspended benthic FPOM with the sample. Pour the water through the mesh and into the carboy until filled. This sample will be used to estimate FPOM concentrations of <250 μm particle size. If the sample is to be used for particle size analysis and seston concentration likely is low (e.g., during winter sampling or in streams with little allochthonous inputs), it is advisable to fill a second or even third carboy for sample processing.
3. Label the carboy(s) by sampling site and sample number and transport to the laboratory for processing. If the sample is to be used for particle size analysis, laboratory filtration should be completed within a few hours following collection of field samples.
4. For the *Miller net* sample (steps 4–11): record initial reading of flowmeter in the Miller net prior to putting sampler into the stream. Record start time of sampling (optional).
5. Suspend the Miller net in the water column with the front opening completely submersed. Use a tether line on the front of the sampler to secure the sampler in place (e.g., tied to a bridge rail, overhanging limb, or held by the operator if stream velocity is not prohibitive).
6. Sampling time will vary depending on the amount of suspended material. Generally, 10–30 min is an adequate sampling period, but more time might be needed if seston concentrations are low and little or no material is readily visible in the collection net at the end of sampling. Conversely, less time is needed

if seston loads are heavy. If larger materials are present in the water column (i.e., leaves or sticks), check to make sure the opening of the Miller net or flowmeter is not obstructed.

7. Upon completion of sampling, record final flowmeter reading and (optionally) stop time of sampling.

8. Wash material from the collection net into a 1-mm mesh sieve nested over a 250-μm mesh sieve. This separates out CPOM, which would otherwise result in an overestimate of FPOM during sample processing.

9. Wash material retained on the 250-μm sieve into a sample bag or sample jar. This collection will be used to estimate FPOM concentrations of the particle size class >250 μm. Label bag or jar by sampling site and sample number, and transport to the laboratory for processing. If the sample is to be used for particle size analysis, laboratory filtration should be completed within a few hours following collection of field samples.

10. To estimate the volume of water filtered through the Miller net, the following information is needed: radius (r in m) of front opening and distance (d in m) of water filtered as measured by the flowmeter.

11. The volume (V in L) filtered is calculated by the equation:

$$V = r^2 d * 1000 \qquad (12.1)$$

12. If elapsed sampling time (t) was recorded in seconds, a measure of velocity (*v* in m/s) can also be made:

$$v = d \div t \qquad (12.2)$$

Although velocity is not crucial to estimations of seston concentration, it is an additional and easily-calculated physical parameter by this method. A data sheet for recording and calculating water volume and velocity information using Miller-type tow nets is presented (see Table 12.1).

2. Protocol for Seston Sampling in Large Rivers

1. The protocol described is for bridge sampling. Measure river width below the bridge with a tape measure.

2. Divide the river width measurement by 11 and use the result to determine 10 equidistant points across the river. Using an erasable marking pen, clearly mark and number these 10 points on the bridge railing. These will be the locations of the sampling verticals.

3. Measure the distance from bridge rail to the water surface and from bridge rail to river bottom at each vertical; the difference between these distances is total depth at each vertical.

TABLE 12.1 Field Collection of Seston Using Miller-Type Tow Nets.

Date _____   Observers _____

Stream and site _____   Sampling location _____

_____   Stream stage condition _____

Net mesh size _____ μm   **(A)** Diameter of net opening _____ m   **(B)** Radius of net opening **(A ÷ 2)** _____ cm   **(C)** pi = 3.1416

(D) Flowmeter conversion to meters _____   **(E)** Volume conversion **(B^2 × C ∗ 1000)** _____

_____

_____

Sample & Filter Number	(F) Initial Flowmeter Reading	(G) Final Flowmeter Reading	(H) Elapsed Time (sec)	(I) Elapsed Flow (G − F)	(J) Water Filtered (m) (I × D)	(K) Volume Filterered (L) (J × E)	(L) Velocity (m sec^{-1}) (J ÷ H)
____	____	____	____	____	____	____	____
____	____	____	____	____	____	____	____
____	____	____	____	____	____	____	____
____	____	____	____	____	____	____	____
____	____	____	____	____	____	____	____
____	____	____	____	____	____	____	____
____	____	____	____	____	____	____	____
____	____	____	____	____	____	____	____
____	____	____	____	____	____	____	____
____	____	____	____	____	____	____	____

4. The number and depth of collections for point samples at each vertical is determined from water depth of individual verticals as follows:

Water Depth	Sampling Depth (measured from surface)
≤1 m	60% of water depth
1–3 m	20 and 80% of water depth
≥3 m	20, 60, and 80% of water depth

5. Lower Kemmerer or Van Dorn bottle to appropriate depth, close, and retrieve sampler. Filter samples through a 1-mm mesh sieve or bolting cloth into milk jugs to remove CPOM. Mark each jug as to collection site, vertical, and sample depth.
6. Transport samples to the laboratory to determine seston concentrations (see "Standard Processing Protocols" below).
7. To determine mean seston concentration, first calculate mean concentration for each vertical and then calculate the mean of all 10 verticals.

3. Standard Processing Protocols

1. Set up a microfiltration unit consisting of a filter holder, base, and funnel that can accommodate 47- or 50-mm diameter filters. The microfiltration unit is seated on a 2- to 4-L capacity filtering flask connected with vacuum tubing to a vacuum pump. Filters are 47- or 50-mm diameter glass-fiber filters (GFFs), preashed, and preweighed.[1]
2. For carboy and jug samples, vigorously shake carboy or jug to resuspend seston. Pour a 1–4 L aliquot of the sample into a graduated cylinder. Record the volume of sample used.
3. Pour the aliquot into the microfiltration funnel and draw down onto a GFF under vacuum. Volume required will vary depending on seston concentration in the aliquot. In general, volume should be sufficient to produce a clearly visible layer of seston on the GFF.
4. Rinse the microfiltration funnel with distilled/deionized, prefiltered water to ensure seston particles are not adhering to funnel. Remove GFF from the microfiltration unit with blunt forceps and return to its labeled aluminum square.
5. Repeat steps 2, 3, and 4 until three replicate aliquots have been filtered for each sample.
6. For Miller net samples, thoroughly wash seston out of the sample bag or sample jar and into the microfiltration funnel using distilled/deionized, prefiltered water and draw down onto a GFF under vacuum. Rinse microfiltration funnel and remove GFF as above.
7. To determine dry mass, seston samples and GFFs should be oven-dried (50°C for 24 hr), desiccated (24 hr), and weighed on an analytical balance.
8. To determine ash mass, dry-weighed seston samples and GFFs should be ashed in a muffle furnace (500°C for 0.5 to 1 hr), rewetted with distilled/deionized water to restore waters of hydration (Weber 1973), oven-dried (50°C for 24 hr), desiccated (24 hr), and weighed on an analytical balance.
9. Masses obtained provide measures of ash-free dry mass (AFDM) or organic seston (dry mass − ash mass) and inorganic seston (ash mass). Masses from >250 μm and

---

[1] Filters should be free of binder such as Gelman type A/E or equivalent. Labeled squares of aluminum foil are useful for maintaining individual preashed and weighed GFF filters.

$<250\,\mu m$ fractions need to be mathematically combined if samples were collected using Protocol 1 (above). Seston concentrations may be reported directly as mg or g of seston per sample volume. However, it is preferable to standardize units to either mg/L or g/m^3.

4. Particle-Size Separation Protocols

1. Set up the wet filtration system consisting of a funnel, a series of stacked sieves in decreasing size order, a base to attach the sieve stack to a filtering flask, and an electric vacuum pump connected to the flask with vacuum tubing (Figure 12.2). A large-capacity ($\geq 4\,L$) vacuum flask should be connected between the stacked sieves and the vacuum. For carboy samples, the largest sieve size should be $250\,\mu m$, which should be the smallest sieve size for Miller net samples.
2. For carboy samples (steps 2–8), vigorously shake the carboy to resuspend seston. Slowly pour the sample from the carboy into the funnel of the filtration system. The volume of water required will vary depending on seston concentration in the sample. In general, it will take the entire volume of the carboy but somewhat less if seston loads are high. Under conditions of low seston concentrations, several carboys may be needed to obtain adequate samples.
3. Filtration will require turning off the vacuum periodically to empty the filtering flask to avoid pulling water into the vacuum pump. Carefully disconnect the stack and base from the flask and *record the volume of water* in the flask prior to discarding. Make sure at least 3 L of filtrate is retained to measure ultrafine seston. Reassemble the system and continue filtration.
4. When filtration is complete, compute and record the total volume of water filtered through the system. Disassemble the wet filtration system, arranging sieves such that size fractions are clearly denoted.
5. Set up a microfiltration unit as described in step 1 of "Standard Processing Protocols".
6. Starting with the largest sieve (i.e., $250\,\mu m$), wash retained material into the funnel of the microfiltration unit with distilled/deionized, prefiltered water and draw down onto a preashed, preweighed GFF under vacuum. Rinse funnel to ensure seston particles are not adhering. Remove GFF from the microfiltration unit with blunt forceps and return to its labeled aluminum square.
7. Repeat Step 6 for the next smaller sieve size, carefully recording sieve sizes and corresponding GFF identification numbers. Continue until material from all sieves has been drawn down onto separate GFFs.
8. Filter reserved ultrafine seston filtrate onto a GFF. Volume required will vary depending on seston concentration in the aliquot. In general, volume should be sufficient to produce a clearly visible layer of seston covering on the GFF. Record the volume of filtrate used.
9. Process GFFs and seston samples as described in steps 7 and 8 of "Standard Processing Protocols".
10. For Miller net samples (steps 10–15), resuspend sampled material in distilled/ deionized, prefiltered water and pour into the funnel of the wet filtration system. Carefully wash out sample bag or jar into funnel to ensure all material is recovered.
11. Draw down material into the stacked sieve column under vacuum while rinsing funnel with distilled/deionized, prefiltered water to ensure seston particles are not adhering.

12. When filtration is complete, disassemble the wet filtration system, arranging sieves such that size fractions are clearly denoted.
13. Set up a microfiltration unit as described in step 1 of "Standard Processing Protocols".
14. Starting with the largest sieve, wash retained material into the funnel of the microfiltration unit with distilled/deionized, prefiltered water and draw down onto a preashed, preweighed GFF under vacuum. Rinse microfiltration funnel and remove GFF as above.
15. Repeat step 13 for the next smaller sieve size, carefully recording sieve sizes and corresponding GFF identification numbers. Continue until material from all sieves has been drawn down onto separate GFFs.
16. Process GFFs and seston samples as described in steps 7 and 8 of "Standard Processing Protocols".
17. Seston concentrations can now be determined as mg per sample volume for individual particle sizes. Since water volume filtered to obtain samples differs between carboy and Miller net samples, concentrations must be converted to a standard unit (e.g., mg of seston/L) prior to comparison.

5. Optional Experiment a: Seston Export

1. Export, or total transport of seston, requires knowledge of stream discharge at the time of seston sampling (see Chapter 3 for methods of determining discharge). Estimates of total export are made by weighting seston concentration (mass per unit volume) by discharge (volume per unit time) to determine export or total transport (mass per unit time). Provided you have the necessary data, this is easily accomplished by multiplying total seston concentration (mg AFDM/L) $\times 1000 =$ mg AFDM/m^3. The product is multiplied by discharge (m^3/s) to estimate mg of FPOM exported per second.
2. One advantage to sampling large rivers is that these systems are routinely gaged and information such as mean daily discharge (in ft^3/s or m^3/s) is easily obtainable from United States Geological Survey's *Water Resources* data book published annually for each state (usually found in government publications sections of most libraries, or on the world wide web at http://waterdata.usgs.gov/nwis). Daily loads of transported seston (seston export) for large rivers can then be estimated by adjusting for units of measure and multiplying seston concentration by mean daily discharge.
3. Other methods of estimating export include the use of rating curves (see Chapter 7; Cummins *et al.* 1983, Webster *et al.* 1990), thus incorporating some aspect of discharge to estimate POM concentrations. However, discharge and POM concentrations are generally poorly related (Bilby and Likens 1979, Gurtz *et al.* 1980, Cuffney and Wallace 1988). These studies indicate that infrequent sampling and poor rating curves are not good predictors of POM export.
4. Another method for continuous export measurements involves Coshocton proportional samplers, which are only suitable for small streams and require more elaborate instrumentation (Cuffney and Wallace 1988, Wallace *et al.* 1991).

6. Optional Experiment b: Seston Sampling During Storms

1. In small streams with quickly fluctuating ("flashy") discharge, the bulk of the total suspended material is carried during the rising hydrograph of storms

(e.g., Gurtz *et al.* 1980, Wallace *et al.* 1991, Webster *et al.* 1990). Sampling these events can be difficult due to their unpredictable timing and short duration. Although seston sampling for particle-size analysis can be conducted under such conditions, it is extremely labor intensive and for our purposes we will only examine total seston concentrations under conditions of baseflow and rising and falling hydrographs. As storms are largely unpredictable, this will require access to a stream located near your laboratory that can be readily sampled. Small, gaged streams are ideal for this purpose. If none are available, see Chapter 3 for stream gaging methods.

2. In some cases a meter stick anchored vertically to an area where the cross-sectional profile can be measured will suffice as a gage. Record the water height on the meter stick with each sample taken during the storm. Use standard processing procedures to calculate total dry mass, ash, and AFDM concentrations for these samples. You may wish to repeat these measurements over a several-day period if no storms occur. You should have a series of 10 to 15 clean bottles with caps (1- to 2-L capacity) for sampling as storms approach as well as a supply of preashed and preweighed GFFs. Start your storm sampling sequence prior to the first rainfall, if possible. Clearly record the time and water height for each subsequent sample as stream turbidity increases on the rising hydrograph during the storm. Brief and intense summer thundershowers are ideal for this purpose; however, severe electrical storms can be dangerous and be sure not to seek shelter under tall trees between sampling intervals. Although timing is tricky for such storms, you should attempt to sample over a period that provides a series of samples taken over both the rising and falling hydrographs. In the laboratory, process each sample separately, clearly labeling each filter from the sequential samples. Determine total dry mass, ash, and AFDM (mg/L) for each sample in the storm sequence.

B. Basic Method 2: Fine Benthic Organic Matter

1. Protocols for Field Collection of FBOM

1. Prepare a substantial amount of filtered stream water: pour stream water into a carboy or other large, clean vessel through a 250-μm mesh sieve.
2. Select sites that are characteristic of either depositional or erosional stream habitats (see Site Selection above). Place a graduated barrel (or large bucket) and paddle in close proximity to the sampling site.
3. With minimal disturbance to the substratum, force the sampling corer into the substratum. The core should be ≤22 cm dia. made of steel or PVC pipe. For cobble-riffle and bedrock-outcrop habitats, wrap a cloth towel around the outside base of the corer once it is in place to form an effective seal with the substratum.
4. Remove material from within the corer with either a plastic cup or hand-powered diaphragm pump (the latter works more efficiently on hard-bottomed substrata). Pass removed material through nested 1-mm and 250-μm mesh sieves that are positioned over the graduated barrel to retain water passing through the smaller mesh.
5. In riffle areas, cobbles inside the corer should be thoroughly brushed and disturbed while pumping; bedrock substratum also should be thoroughly brushed.
6. Once water has been removed from inside the coring device and the substratum cleaned of fine particles, thoroughly wash the sieves with filtered stream water,

retaining material passing through the bottom sieve in the graduated barrel. Measure and record water volume in the barrel.

7. For the <250 μm fraction, stir water in the barrel thoroughly with the paddle and remove a subsample of the agitated water (0.2 to 1 L, depending on the concentration of particles). Store subsamples in either separate bottles or large, self-closing plastic bags. Three replicate subsamples (stirring before each subsample) are desirable for each sample.

8. For the >250 μm fractions, discard material retained on the 1-mm mesh sieve, which is the CPOM fraction of the sample. Wash material retained on the 250-μm mesh sieve with filtered stream water into a suitable container (e.g., large plastic bag or wide-mouth bottle), and clearly label this container and the subsamples.

9. Repeat the sampling procedure for all targeted habitats (i.e., erosional and depositional areas).

2. FBOM Processing Protocols

1. For the >250 μm fractions (steps 1–5), wash contents of the sample container with tap water into a large pail and resuspend in water.

2. Pour the resuspended material through nested 500-μm and 250-μm mesh sieves. Allow time to drain samples thoroughly and transfer material to separate, labeled paper bags.

3. Oven-dry material and bags at 50°C to constant weight (24 hr to several days, depending on sample size). Place bags in a desiccator for 24 hr.

4. Remove material from bags and weigh on a top-loading balance to determine dry mass.

5. Ash material at 500°C (small, heavy-gauged, aluminum baking pans work well for this purpose), and reweigh to obtain AFDM for the 250 to 500 μm and 500 μm to 1.0 mm size fractions.

6. For the <250 μm fraction (steps 6–11), set up a microfiltration unit as described in step 1 of "Standard Processing Protocols".

7. Individually pour each of the three replicate subsamples into separate 1-L graduated cylinders and record the subsample volumes.

8. Pour the first subsample into the funnel of the filtration unit. Wash any material clinging to the subsample bag or graduated cylinder into the funnel with distilled/deionized, prefiltered water. Draw material down onto a GFF, washing sides of funnel with distilled/deionized prefiltered water. Remove filter with blunt forceps and return to aluminum square.

9. Repeat steps 7 and 8 for remaining replicates.

10. Dry, weigh, ash, and reweigh FBOM samples and GFFs following steps 7 and 8 of "Standard Processing Protocols".

11. AFDM of the 0.45 μm to 250 μm size fraction is estimated as the mean of the following quantity calculated for each of the three subsamples:

$$\text{AFDM} = (\text{barrel volume} \div \text{subsample volume}) \times \text{subsample AFDM} \qquad (12.3)$$

12. FBOM quantity is normally expressed as g AFDM/m^2 of stream bottom. This requires you to know the area of your sampling device (in cm^2). Use the following equation for FBOM standing stocks estimated for each size fraction to express your results:

$$\text{g AFDM/m}^2 = (\text{mg AFDM} \div 1000) \times (10{,}000 \div \text{cm}^2 \text{ of area sampled}) \qquad (12.4)$$

The g AFDM/m^2 for each size fraction are summed to obtain total FBOM standing crop (in AFDM) in your sample.

C. Advanced Method 1: Linkages of Sestonic FPOM to the Biota

1. Field Release and Larval Collection

1. In the field, thoroughly mix charcoal with stream water in one or two large pails until no more charcoal remains on the surface of the water, to form a dense slurry of suspended charcoal (some continuous stirring even during release may be required to ensure suspension).
2. Position members of the team on either side of the black fly aggregation, being careful to minimize disturbance.
3. At a location 1–2 m upstream of the black fly aggregation, slowly pour the slurry back and forth across a 0.5–1.0 m width of stream, ensuring that the water passing over the larval aggregation is darkly stained with charcoal particles. Pour slowly to ensure that the contents of the pail are not released as a massive instantaneous dosage. (A beaker can be used for removing the slurry from the bucket and releasing the mixture in the stream.)
4. The release should take 1 to 2 min. Record the starting and ending time of the slurry release. Note the width of the slurry passing over the aggregation and the lateral boundaries of the slurry (flagging attached to wire stakes may be useful for this purpose) and keep larval collections within boundaries.
5. Collect larvae at 10-, 20-, and 30-min intervals following slurry release.
6. Use collecting forceps to pick larvae and place in a vial prelabeled with the appropriate time interval and half-filled with 70% ethanol. Collectors should strive to sample a range of larval sizes at each period. Sampling should continue for about 1 min after each 10-min interval. Following each collection period, check all vials to ensure that the time interval is correctly indicated.

2. Laboratory Analysis

1. Separate vials into specific time intervals and work with larvae from only one interval at a time to avoid confusion.
2. Starting with larvae collected 10 min after charcoal release, use a dissecting microscope fitted with an ocular micrometer to divide black flies into size classes to the nearest 0.5 mm. Keep size classes separate.

3. For each size class, use the ocular micrometer to measure the distance from the posterior end of head to the tip of the abdomen on each larva. Record data as Distance x.
4. Using the point of the jeweler's forceps, carefully split open the larval integument from below the head to the tip of the abdomen.
5. With two pairs of jeweler's forceps, gently tease the gut out of the body cavity, keeping the head attached to the gut.
6. Measure with the ocular micrometer the distance from the posterior end of the head to the charcoal band in the gut. Record data as Distance y.
7. Repeat this procedure until all larvae from each size class and all three time intervals have been measured.
8. Upon completion of measurements, you should have recorded the following information for each larva collected:
   a) Collection interval (10, 20, or 30 min);
   b) Larval length (mm);
   c) Distance y in mm (posterior end of head to charcoal band);
   d) Distance x in mm (posterior end of head to tip of abdomen);
   e) Ratio W (Distance y ÷ Distance x).
   f) Plot the ratio of W (*y*-axis) against the larval length (*x*-axis) for each larva examined at the 10-min interval. Repeat this process for each larva measured for the 20-min interval, then the 30-min interval. You should be able to regress the values for the ratio of W and larval length for each time interval.

IV. QUESTIONS

A. Seston

1. What is the total organic seston (in mg AFDM/L) concentration in your stream?
2. Based on your measurements of individual size classes, what sizes are the most abundant in terms of total organic seston in transport?
3. Suppose you repeat these measurements in smaller headwater streams or a larger downstream river. Would you expect the same results? Why or why not?
4. How does seston concentration vary with stream depth? with distance from the thalweg?
5. Does seston quality (in terms of organic:inorganic ratio) change with distance from the thalweg? If so, can you hypothesize as to why this change occurs?
6. If seston concentrations are available for two rivers or sites, or the same river in different seasons, compare estimates of seston export between rivers, sites, or seasons. How do they compare and can you suggest any mechanisms to account for differences?
7. (*Optional Experiment a*) Convert seston concentration data into an estimate of total seston export. What source did you use for discharge data? Can you predict seasonal patterns of seston export for your system based on what information you have on discharge and seston concentrations?
8. (*Optional Experiment b*) How did the dry mass, AFDM, and ash concentration change over the rising and falling hydrograph of the storm? If you are working in a gaged stream it will be useful to plot each sample concentration against discharge at the time the sample was collected. If not you can plot each sample

against water depth measured on the meter stick as a rough estimate of relative discharge for each sample.

9. (*Optional Experiment c*) At what stage of the storm sampling sequence was maximum and minimum seston concentrations reached? How do you explain your results?

10. (*Optional Experiment d*) Based on your sampling results during the storm, what problems do you see with calculating organic matter export for stream ecosystems? How does this influence organic matter budgets for a given stream reach?

B. Fine Benthic Organic Matter

1. How do FBOM particle-size distributions and total FBOM standing crops compare between erosional and depositional habitats?
2. Hypothesize as to the specific physical characteristics in each habitat which account for differences in FBOM standing crops.
3. Given differences in FBOM particle-size distribution and standing crops, what are your hypotheses concerning the relative functional structure of the benthic macroinvertebrate community in each habitat? (See Chapters 20 and 25 for information concerning benthic community functional structure.)

C. Linkages of Sestonic FPOM to the Biota

1. Do black fly larvae display any tendency to select food particles based on type of food available? Give reasons for your answer.
2. Black fly larvae have been described as feeding nonselectively on particles $<300\,\mu m$ in diameter. Based on your analyses of seston particle sizes, what significance do you attach to this observation with respect to particle size availability in lotic habitats?
3. For a specific time interval—say, 10, 20, or 30 minutes—following charcoal exposure, is there any difference in gut passage times for larvae of different size classes? If so, what differences did you detect? What does the ratio of W versus larval length illustrate about gut passage times?
4. What is your best estimate of gut passage time for black fly larvae of different size classes? Did you notice any difference in charcoal bands after 30 minutes? How do you account for differences after longer time intervals (see Wotton 1980)?
5. What do you see as the "ecological role" micro-filtering collectors such as black flies play in stream ecosystems? Explain your answer.

V. MATERIALS AND SUPPLIES

Letters in parentheses indicate in which Method (1, 2, or 3) the item is used.

> Aluminum squares (1, 2). Approximately 60 mm side length and numbered to facilitate filter identification.
> Balance (1, 2), analytical.
> Balance (2), top-loading.
> Bags, paper (2).

Bags, plastic (1, 2). Self-closing (e.g., Ziploc® or Whirl-Pak®).

Bottles (2), wide-mouth, capped, 1 to 2-L capacity.

Buckets, 10- to 15-L capacity (3).

Charcoal, fine-powered (3). Optional: fine powdered fluorescent pigments can be substituted for charcoal (e.g., Miller *et al.* 1998). These are more expensive than powdered charcoal but easier to locate in the gut, especially for black fly larvae with heavily pigmented integuments. They also glow when exposed to a black light source, such as a mineral light used by geologists. One source of such pigments is Radiant Color, 2800 Radiant Ave., Richmond, CA 94804. Type P-1600 (avg. particle size $= 5\,\mu$m) manufactured by Radiant Color have the added advantage that specimens can be mounted on glass slides without interference from the many solvents used in mounting.

Corer (2). Handheld or stove-pipe with inside diameter 22.6 cm or greater made of steel or PVC pipe that can be forced into the substratum.

Cup, plastic (2). For sampling depositional areas.

Desiccator (1, 2). With $CaSO_4$ desiccant.

Filters (1, 2). 47 or 50 mm glass-fiber filters without binder (e.g., Gelman type A/E, Whatman GFF, or equivalent). Prior to use, filters are ashed in a muffle furnace (500°C for 0.5 to 1 h), rewetted with distilled/deionized water to restore waters of hydration, oven-dried (50°C for 24 h), desiccated (24 h), and preweighed on an analytical balance. Store glass-fiber filters on labeled aluminum squares in a desiccator.

Flags (3). (optional) Attached to wire stakes.

Forceps, jewelers (3), blunt (1, 2).

Furnace, muffle (1, 2).

Graduated container (2). Large pail or vinyl trash can marked for the volume of water at various depths.

Graduated cylinders, 1-L capacity (1, 2).

Jugs, approximately 1-gal capacity (2). Milk jugs are good for this purpose as they are inexpensive and many are needed.

Microfiltration unit (1, 2). Includes:

> 47- or 50-mm filter base, holder, and funnel
> filtering flask, 2 to 4 liter capacity
> vacuum pump
> vacuum tubing
> blunt forceps

Marker, permanent ink (1, 2, 3).

Microscope, dissecting, binocular (3). Fitted with an ocular micrometer.

Notebook, field (1, 2, 3). Waterproof pages.

Oven, drying (1, 2).

Paddle, canoe (2, 3).

Pump (2). Handheld, diaphragm-type, for sampling erosional areas.

Sampler, point (1). Includes:

> Kemmerer or Van Dorn sampling bottle
> weighted messenger
> tether line marked in 0.5-m increments.
> line depressor

Sampler, Miller-type tow-net (1). Includes:

> sampler body with slightly tampered front (reduction fitting)
> collecting net, 250 μm
> catch bucket
> flowmeter
> tether line

Sieves, standard testing (1, 2). Nestable, with mesh sizes of 250 μm, 500 μm, and 1.0 mm.
Stopwatch (1, 3).
Tape, measuring (1). 10 to 50 m marked in 0.5-m increments.
Wash bottles (1, 2).
Wet filtration unit (2). Includes:

> top funnel
> nestable sieves of decreasing mesh sizes. Examples: 500 μm, 250 μm, 100 μm,
>   50 μm, and 25 μm
> filtering flask, 2 to 4 Liter capacity
> Teflon® gaskets
> vacuum pump
> vacuum tubing

Vials, 1 dram with stoppers (3). Vials should be half-filled with 70% ethanol and
    prelabeled to indicate 10, 20, and 30 minute collection intervals.
Water, distilled/deionized and prefiltered through glass-fiber filters (1, 2).

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CPOM Transport, Retention, and Measurement

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I. INTRODUCTION

Coarse particulate organic matter, or CPOM, in streams is defined as any organic particle larger than 1 mm in size (Cummins 1974). CPOM can be further divided into wood and nonwoody material (Cummins and Klug 1979), both of which are considered in this chapter. Wood includes all size classes from branches to entire trees that fall into stream channels. Wood can form impressive *dams* or accumulations across stream channels, which have important ecological functions (Bilby and Likens 1980). The nonwoody fraction includes *allochthonous* materials donated by riparian vegetation (e.g., leaves, needles, fruits, flowers, seeds, insect frass) and *autochthonous* materials produced within the stream (e.g., fragmented aquatic plants, dead aquatic animals). Smaller materials, including fine particulate organic matter (1 mm > FPOM > 0.45 μm) and dissolved organic matter (DOM < 0.45 μm), are discussed in Chapters 11 and 12.

Allochthonous CPOM is a major energetic resource for stream ecosystems, providing a large proportion of the fixed carbon in small streams of both deciduous and coniferous forests and a significant input to larger streams and rivers (Vannote *et al.* 1980, Cummins *et al.* 1983). CPOM that enters streams is *transported* downstream by the unidirectional flow of lotic ecosystems, with very few mechanisms for upstream movement. Trapping of this material is therefore essential for the subsequent microbial colonization that normally precedes consumption by shredding macroinvertebrates (Cummins and Klug 1979).

The process of deposition and trapping, termed *retention*, provides the critical link between input and the long-term storage and processing of CPOM.

The retentive capacity of streams for CPOM is a function of hydrologic, substrate-related, and riparian features (Speaker *et al.* 1984). High *roughness* of the channel (e.g., large substrate particle size, streambed heterogeneity, abundant wood), combined with certain hydraulic conditions (e.g., presence of backwaters, interstitial flow), tends to increase the CPOM trapping efficiency of stream reaches. Wood dams are particularly important retention structures (Bilby 1981, Smock *et al.* 1989). Young *et al.* (1978) noted that the probability that a particle in transport will be retained is a function of the "active" entrainment efficiency of that particle size by a channel obstacle (e.g., rock, log, root, etc.) and the density of those obstacles within the channel. Particles also will be retained "passively" when current velocity is less than the velocity required to keep the particle moving in the water column or along the streambed (Jones and Smock 1991) and thus the particle "settles". Retention (R) can thus be expressed as a probability function:

$$P(R) = f(E, N, V) \tag{13.1}$$

where E = entrainment efficiency by channel obstacles, N = obstacle density in the channel, and V = critical velocity required to transport a particle. If an organic particle is retained, it subsequently will either decompose, be consumed, or, if flow conditions change, be dislodged and transported further downstream (Speaker *et al.* 1984).

Wood is a major roughness element in streams that influences channel morphology, decreases the average velocity within a reach, and physically traps material in transport (Lamberti and Berg 1995, Gregory *et al.* 2003a, Montgomery *et al.* 2003, Mutz 2003). The amount of wood (W) in a stream channel is a function of the lateral input from the riparian forest (F_{in}), transport into a reach from upstream (T_{in}), biological decomposition (D), mechanical abrasion (A), and transport out of the reach to downstream areas (T_{out}):

$$W = f(F_{in}, T_{in}, D, A, T_{out}) \tag{13.2}$$

Causal factors for amounts of wood measured locally in a stream reach cannot be determined from simple inventories. Long-term measurements of input rates, sources, breakdown rates and transport can provide the information necessary to interpret local wood abundance, but such extensive studies are costly and time consuming. An alternative to measuring all physical and biological processes that affect storage of wood is simulation modeling.

At least 14 models of wood dynamics have been developed for different regions (Gregory *et al.* 2003b). Some models address specific processes, whereas others provide quantitative representations of riparian forest growth and mortality, input processes, disturbance processes, and in-channel processes that modify wood storage. Studies of large wood and its influence on CPOM retention may require use of a regionally relevant model of stream wood dynamics (e.g., see example from the U.S. Pacific Northwest later

in this chapter). Several models offer the ability to alter the critical parameters in the model so that the user can adapt the model to different tree species, flow regimes, and channel structure.

In this chapter, we describe a quantitative field method to assess the CPOM retention efficiency of a specific stream reach. The method is most easily used in small streams (orders 1–4) but can be adapted for larger streams and rivers. The approach is intended not only to measure retention but to relate retention to hydraulics, streambed roughness, channel geomorphology, and riparian zone structure. Because large wood in the channel is central to CPOM retention, we also present two methods to quantify the abundance of large wood in stream reaches. Finally, we illustrate the application of a publicly available model to explore the dynamics of large wood under differing forest management scenarios. Our specific objectives are to (1) introduce the concept and importance of organic matter retention; (2) demonstrate how to measure retention, analyze data, and calculate indices of retention; (3) illustrate the utility of retention measurements for assessing stream channel condition; (4) describe the direct count and line-intersect methods for estimating large wood abundance in stream channels; and (5) demonstrate the use of a simulation model for wood input and dynamics.

Note that this chapter focuses on short-term trapping of CPOM and does not consider its long-term storage or breakdown except in a modeling context (but see Chapters 30 and 31). Also, we do not describe benthic sampling of CPOM, sometimes referred to as coarse benthic organic matter (CBOM), because detailed methods are presented in Chapter 31. However, benthic sampling of CPOM can augment the exercises below because storage is the ultimate expression of transport and retention.

II. GENERAL DESIGN

In practice, lotic retention can be viewed as the difference between the number of particles in transport at a given point in the stream and the number still in transport at some known distance downstream (Speaker *et al.* 1984). Retention is most easily measured by releasing known numbers of readily distinguishable particles into the channel. To compare different stream reaches within a study, the experimental approach must be standardized for type and number of particles released, length of experimental reach, and duration of the retention measurement. Many types of CPOM have been released into streams, including leaves (Speaker *et al.* 1984, Ehrman and Lamberti 1992), paper shapes (Webster *et al.* 1994), plastic strips (Bilby and Likens 1980, Speaker *et al.* 1988), wood dowels (Ehrman and Lamberti 1992), and even fish carcasses (Cederholm *et al.* 1989). In general, we believe that it is preferable to release natural (decomposable) materials into streams because particle retrieval is almost always less than 100% and because analogs (e.g., plastic items) may not behave the same as natural materials. In this chapter, we will demonstrate retention of leaves and small wood, but other materials significant to the specific stream can be substituted. For example, fruits are significant CPOM inputs in many tropical streams. We will also describe methods for quantifying the abundance of large wood, which provides important sites for CPOM retention, and modeling wood dynamics with simulation approaches.

A. Site Selection

The selection of a study stream in which to conduct this exercise may be influenced by logistical considerations. In general, wadeable third- to fourth-order streams are ideal. Very small streams at low flow have low transport, and the method described in this chapter is difficult (and can be dangerous) to conduct in large rivers. In general, however, this method can be scaled to a wide variety of stream sizes. Within the study stream, at least two reaches with contrasting channel features should be selected by the research coordinator. Ideally, one reach would have a relatively simple channel (straight, low roughness, limited hydraulic diversity, sparse wood) whereas the other reach should have a complex channel (sinuous, high roughness, diverse hydraulic conditions, abundant wood).

Length of the experimental reach should be scaled to stream size, with length increasing with stream order. As a rule of thumb, start with a stream length that is ∼10 times the wetted channel width. For example, 50 m may be an appropriate length for a second-order stream, 100 m for a third-order stream, and 200 m for a fourth-order stream. Streams of the same size in different settings will have specific retention characteristics. If possible, use a pilot study to adjust reach length such that retention is not less than 10% nor greater than 90% of released particles.

B. Basic Method

Leaves are the major form of nonwoody CPOM input to most streams and their retention is an important ecological process (Webster *et al.* 1999). In retention experiments, released leaves must be distinguishable from leaves found naturally in the channel and should be easy to obtain and manipulate. We have found that, for North American streams, abscised leaves of the exotic Asian ginkgo tree (*Ginkgo biloba*) meet these requirements. The leaves are tough even when wet, their size approximates that of many leaf-types of riparian vegetation, and the bright yellow leaves are easily spotted in the channel. *Ginkgo* trees have been planted worldwide as ornamentals (and very often on college campuses), which usually are male trees because female trees drop unpleasantly pungent fruits in the autumn. Other species of leaves can be substituted depending on their availability and the composition of local riparian vegetation. Released wood similarly must be easy to manipulate, distinguishable from natural wood in the channel, and of a realistic size. We have found that these requirements are met by wood dowels, which can be obtained at hardware stores in a range of diameters and lengths. Dowels, however, have a simpler shape than tree branches and will be a conservative estimator of wood retention. Alternatively, fallen branches can be collected from the site and marked with fluorescent paint to distinguish them from existing wood in the channel. Keep in mind that it is more difficult to standardize branches among releases than dowels.

Physical data from the stream channel should be analyzed according to the level of measurements taken (see Chapters 2–4). At a minimum, the following parameters should be measured for each study reach: discharge, slope, sinuosity, cross-sectional area, planar wetted area, and volume of large wood (using direct counts or the line-intersect method). Retention data for leaves and small wood (using batch releases) should be fit to a negative exponential decay model, from which various indices of retention (e.g., the retention coefficient -k; average particle travel distance 1/k) can be calculated. Metrics for individual particle releases can be generated using simple statistics. If desired, CPOM releases can be conducted over longer periods of time, or at different seasons and discharges, to develop relationships between retention and stream temporal dynamics (e.g., Jones and Smock 1991, Webster *et al.* 1999).

C. Advanced Methods

Several advanced exercises involving additional sophistication, time, and facilities are also presented in this chapter. These are "research-level" approaches suitable for incorporation into published papers. First, an inventory of leaf and dowel entrapment in the channel can be performed after the release to better describe the pattern of retention and to quantify entrapment by specific benthic features. Second, we describe how basic hydraulic features of the channel can be described with slug releases of conservative solutes into the stream. Third, the dynamics of large wood will be modeled with simulations using a publicly available computer model. If desired, different levels of physical measurement of the channel can be performed (see Chapters 2–4), although these are not presented in this chapter.

CPOM retention often is correlated with *hydraulic retention* (i.e., the retention of water within a reach). Hydraulic retention and discharge can be estimated by releasing a tracer, such as fluorescent dye, as a "slug" into the channel and measuring water movement and dilution through the reach. Discharge calculated from tracer releases and more conventional approaches can be compared (see Chapter 3). The use of tracers that are more conservative than dyes (e.g., chloride) is described in Chapters 8 and 10, along with more sophisticated continuous injections than are presented here. If dye slug releases are conducted, various hydraulic parameters (e.g., discharge, nominal transport time) can be calculated from a plot of dye concentration over time at a downstream sampling site.

III. SPECIFIC METHODS

A. Basic Method: CPOM Transport and Retention

Laboratory Preparation

1. In the autumn, collect several thousand abscised leaves of an exotic tree, such as *Ginkgo biloba*, or other readily identifiable species. Air-dry the leaves by spreading them over screens, netting (seines work well), or even on the floor. Leaves can be stored dry in black garbage bags for a considerable length of time. Alternatively, you can use fresh-fallen leaves if the releases will be performed soon after collection (within days).

2. Count out two batches of 1000 leaves, used to conduct two releases in a third-order stream. Smaller or larger streams may require fewer or more leaves, respectively. The actual number of leaves is less important than knowing exactly how many are released.

3. The day before the release, soak leaves overnight in buckets of water to impart neutral buoyancy during transport. A soil sieve placed gently over the leaves will help to keep them submersed. Drain most of the water before departing into the field.

4. Obtain 60 wood dowels, each approximately 1.5 cm in diameter and 1 m in length; 50 dowels will be used in a single release, and recycled in subsequent releases. (Note: Other dowel sizes, or wood chips, can be used to test retention of variously sized CPOM.) Alternatively, natural sticks can be collected on site from the riparian zone before the release. These sticks can be marked with a spot of fast-drying spray paint to distinguish them from other wood.

Field Physical Measurements

1. Measure and flag an appropriate length (e.g., 100 m for a third-order stream) of at least two stream reaches differing in channel complexity, large wood abundance, or some other relevant feature. Stretch a meter tape along the bank over the length of the reach, with 0 m at the downstream end.
2. Measure major channel features at a level of intensity appropriate to the research objectives. We recommend working in a research team of three people (two making measurements and one recording data). Minimally, measurements should include slope, channel cross section, average width, depth, sinuosity, and substrate composition. Determine discharge using the cross-sectional approach (see Chapter 3). Repeat for each reach.
3. Measure the length (L) and average diameter (D) of all wood contacting the channel and larger than a minimum size (e.g., 1 m L × 10 cm D; Figure 13.1). Note if the wood is part of a dam (i.e., wood accumulation blocking some portion of stream flow). Option 2 to the Basic Method (below) describes an alternate estimation approach if wood is extremely abundant.

Direct Count Method

Large wood volume:
$$m^3/m^2 = \Sigma(\pi L r^2)/A$$

Line Intersect Method

Large wood volume:
$$m^3/m^2 = \pi^2 \Sigma d^2/8L$$
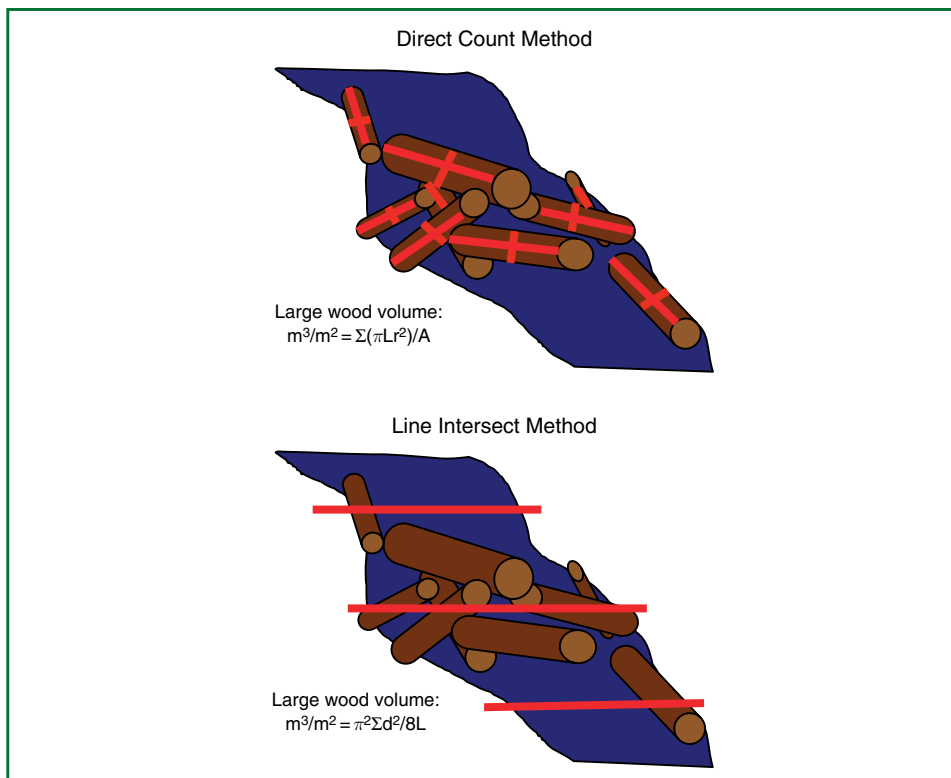
FIGURE 13.1 Methods for measuring large wood in stream channels: Top: direct count method with formula for volume estimation; Bottom: line-intersect method with formula for volume estimation. (Illustrations by J. Miesbauer.)

4. If this method is being used for a class demonstration, prior to the releases briefly discuss channel and riparian features. Have students predict retention for each reach (e.g., percentage of leaves that will be retained).

CPOM Releases

1. Position several researchers at the downstream end of the study reach. Release the leaf batch (e.g., 1000 leaves) at the upstream end of the reach (e.g., 100 m mark) by dispersing leaves over the entire width of the stream channel over a span of about one minute (Figure 13.2A).

2. Collect nonretained leaves at the downstream end of the reach (0 m). Either of two approaches can be used to collect leaves. A beach seine can be stretched across the width of the channel (Figure 13.2D), with the bottom lead-line anchored, without gaps, to the streambed with rocks (in sand-bottom streams, tent stakes can be substituted for rocks). The top of the seine should be held out of the water by attaching it to a taut rope tied to trees on both banks. In strong flows, it may be further necessary to support the top and rear of the seine with wood pieces driven into the substrate. Alternatively, researchers can line up across the channel and collect leaves in transport with handheld dip nets (e.g., D-frame or delta nets). The seine method is more efficient, especially if the number of researchers is low. The

FIGURE 13.2 Photographs of (A) *Ginkgo biloba* leaf release into an Oregon stream, (B) wood dowel collection following release in a Michigan stream at high flow, (C) fluorescein dye release into an Indiana stream, and (D) beach seine stretched across the channel of a northern Alaska stream (anchored to the bottom with rocks) for capturing unretained leaves. (Photos by G. Lamberti.)

individual netting approach results in greater involvement of researchers in actual leaf collection, but some leaves may be missed.

3. Continue collecting leaves for a period of time specified by the coordinator, usually at least 15 minutes and up to 1 hr, or when leaf transport ceases. Release interval should be consistent for all reaches. Count all collected (i.e., nonretained) leaves.

4. Release 50 dowels or sticks into the stream channel and hand-collect nonretained wood at the downstream end of the reach (Figure 13.2B). Count nonretained wood pieces. Retrieve retained dowels from the channel at the end of the exercise.

5. Move upstream to the next reach and repeat the procedure.

Data Analysis

1. Calculate reach physical parameters, such as slope, planar surface, cross-sectional area, mean depth, current velocity, hydraulic radius, sinuosity, and discharge (see Chapters 2–4). These fundamental physical parameters can be related empirically or theoretically to observed retention values.

2. Determine the density (pieces per reach) and total volume (in m^3) of wood in each reach, assuming that a cylinder approximates the geometry of a log such that:

$$volume = \pi L r^2 \qquad (13.3)$$

where L is the length of the piece (m) and r is the radius (m), and then summing for all wood pieces in the reach. Alternatively, you can calculate the volume of wood (m^3) per unit area (A, in m^2) of stream channel:

$$volume\ per\ unit\ area = \Sigma(\pi L r^2)/A \qquad (13.4)$$

3. Fit the leaf and stick retention data to a negative exponential decay model of the form:

$$P_d = P_0 e^{-kd} \qquad (13.5)$$

where $P_0 =$ number of particles released into the reach and $P_d =$ number of particles still in transport at some downstream distance d from the release point. Calculate the slope $-k$ (the instantaneous retention rate) and its reciprocal $1/k$ (the average distance traveled by a particle before it is retained). If particles are not inventoried after the release, then the model will be based on two data points, P_0 and P_d. See Advanced Method 1 for data analysis if particles were re-inventoried in the channel.

Option 1 to Basic Method: Single Particle Release Method

1. As an alternative to batch releases of particles described above, the single-particle release method can be used (Webster *et al.* 1994). Single particles (e.g., leaves, sticks) or artificial analogs (e.g., "Rite-in-the-Rain" field paper, cut into consistent shapes) are released into the channel and individual travel distances are recorded.
2. Release a known number (e.g., 25–50) of visible particles one-by-one into the channel and record the distance traveled and retention structure for each particle. Repeat this procedure in as many stream reaches, or sub-reaches, as desired. Mean travel distances can be compared statistically among reaches using ANOVA, or relationships with stream characteristics such as discharge can be explored with regression (e.g., Webster *et al.* 1994, 1999).
3. This approach is especially useful in highly retentive streams where few or no particles may travel the entire stream reach, thereby invalidating the exponential decay model. However, this method requires relatively high water clarity and shallow depths to follow individual particles for their entire travel.

Option 2 to Basic Method: Line-Intersect Estimation of Large Wood

1. The line-intersect method (LIM) can be used in place of direct counts of large wood in streams having high volumes of wood. For LIM, diameters are measured for all pieces of wood intersecting multiple line transects placed perpendicular to the longitudinal axis of flow (Figure 13.1). LIM was designed to estimate wood on the forest floor (DeVries 1974, Van Wagner 1968), but recently has been adapted for both small streams (Wallace *et al.* 2001) and large rivers (Wallace and Benke 1984, Benke and Wallace 1990).
2. In each study reach, use a tape measure to establish a transect every 5 or 10 m perpendicular to streamflow. Measure the diameter of all large wood pieces intersecting the transect line, using log calipers if available.
3. Compute the wood volume per unit area (m^3/m^2) for each transect using the following equation:

$$volume = \pi^2 \sum d^2 / 8L \qquad (13.6)$$

where d is the diameter of a wood piece (m) and L is the length of the transect line (m) across the stream (Van Wagner 1968). To estimate the average large wood volume (m^3/m^2) for a reach, sum wood volumes for each transect and then divide by the total number of transects.
4. In large rivers or in streams with large amounts of wood, LIM may reduce the effort required to estimate large wood volume. However, LIM may overestimate or underestimate the actual large wood volume, determined by direct counts, depending on stream characteristics and large wood distribution (Wallace *et al.* 2001, Miesbauer 2004).

Option 3 to Basic Method: Long-term CPOM Retention and Transport

1. Conduct CPOM release as in described above, but with one or more of the following modifications.
2. Release dowels over a time span of several weeks or months, depending on the stream and research objectives.
3. Inventory the location of dowels in the channel, but leave them in place and reinventory after varying periods of time. Different sizes of wood also can be released. Year-classes of wood can be marked differently, permitting year-to-year evaluation of transport. Additional releases of leaves and wood can be conducted in different seasons or at different discharges to describe more precisely the temporal dynamics of retention.

B. Advanced Method 1: Importance of Different Retention Structures

1. Conduct CPOM release as in the *Basic Method* above.
2. Inventory the location, number, and retention structure for retained leaves and wood. This is best accomplished by dividing the reach into longitudinal increments of 5 m using the bankside meter tape.
3. Researchers should move up the channel as a single line of observers, perpendicular to flow.
4. Leaves are located and counted within each increment, noting also the retention structure (e.g., rock, wood, bank, etc.; see Table 13.1). Released wood can be inventoried simultaneously and then removed for re-use.
5. The inventory data can be used to refine the exponential model and produce a more accurate estimate of -k, or to fit retention data to an alternate regression model (e.g., linear, power) more appropriate for the specific reach. The inventory most likely will not turn up all of the retained leaves; therefore, it is necessary to normalize inventory data to a percent of total leaves found. Graph the particle transport data for each release, using distance downstream from the release point as the *x*-axis and percent of particles still in transport as the *y*-axis (see Ehrman and Lamberti 1992 for examples). Using a bar diagram, plot the percentage of leaves or dowels retained by specific channel structures in each reach. Describe the longitudinal pattern of retention and identify important retention structures within the channel.

C. Advanced Method 2: Hydraulic Characterization Using Dye Releases

1. Carefully and accurately weigh several batches of fluorescent dye (e.g., 1.0 g of fluorescein powder or rhodamine-WT liquid) into scintillation vials. Number a set of empty scintillation vials from 1 to 100.
2. Qualitatively estimate the amount of dye to be released (1.0 g is appropriate for about 0.25 m^3/s discharge—about a third-order stream). Thoroughly dissolve the dye in a small volume of water (e.g., 1 L). Release the dye slurry at the upstream end of the reach into a constricted, turbulent zone, if available, to ensure rapid mixing

TABLE 13.1	Sample Data Sheet for Inventory of Retained CPOM Particles.

Stream: POM   Date:
                                 Type:

Location: Team:

Reach: Total   Notes:
                                 Released:

Length:

Duration: Total
                                 Captured:

                                 Total
                                 Retained:

Location	Unit	Number of particles retained on structure					
Meter Mark	Riffle or Pool	Rocks	Roots	Backwater	Bank	Wood	Debris Dam
0–5							
5–10							
10–15							
.							
.							
.							
95–100							

of the dye with the stream water (Figure 13.2C). In slower moving water, dispense the dye evenly across the stream channel. Position researchers at the downstream end of the reach with the numbered scintillation vials, a stopwatch, and a notebook.

3. At the downstream end of the reach, the dye concentration curve must be measured accurately by taking water samples as the plume passes through the reach. Commence sampling of water from the *thalweg* (main thread of flow) in the numbered scintillation vials immediately following the release. Sampling frequency and duration will depend on transport time related to stream size, reach length, and channel geomorphology. We recommend that water samples be drawn every 5 seconds as the dye plume passes through the downstream end of the reach. The interval between samples can be lengthened for the trailing edge of the plume. Continue sampling even after visible dye has passed from the reach and until the coordinator indicates to stop (e.g., 5–10 min in a third-order stream). Record elapsed time with each numbered water sample (see Table 13.2).

4. In the laboratory, calibrate a fluorometer with a standard concentration series of the released dye (within the expected dilution range, such as 0.1, 1, 10, and 100 μg/L). Measure and record dye concentration in each water sample using the fluorometer. Dilute samples if dye concentration exceeds your calibration curve.

TABLE 13.2 **Sample Data Sheet for Conducting Hydraulic Retention Study.**

Stream:	Dye:	Date:
Location:	Concentration:	Team:
Reach:	Volume:	Notes:
Length:		

Elapsed Time (min:sec)	Vial Number
0:0	1
0:5	2
0:10	3
.	.
.	.
.	.
5:00	n

5. Dye can be used to calculate several hydraulic parameters, of which we will discuss discharge and transport time. Discharge (Q, in L/s) can be calculated from dye dilution using the equation:

$$Q = VC_u / \int (C_d - C_b)\, dt \qquad (13.7)$$

where V = volume of dye released in L, C_u = concentration of released dye in μg/L, C_d = instream dye concentration at time t in μg/L, and C_b = background fluorescence in μg/L. In general, C_b effectively will be zero and V will equal 1.0 or a very small number compared to stream discharge, and thus can be ignored. The denominator can be calculated by first plotting the measured dye concentration (in μg/L) on the y-axis against time (in seconds) on the x-axis (see Ehrman and Lamberti 1992); then, integrate the area under the dye concentration curve using computer digitation, numerical, or graphical methods (Gordon et $al.$ 1992). Divide Q by 1000 to convert to m^3/s. Nominal transport time (NTT; Triska et $al.$ 1989) is an appropriate measure of hydraulic retention as indicated by a dye slug release. NTT is calculated as the time interval required for 50% of the dye to pass out of the reach. Integration of the concentration curve, starting at the origin and proceeding

until 50% of the total area is found, will yield the NTT. NTT generally increases with reach complexity and the presence of certain channel features, such as large pools or significant interstitial flow.

D. Advanced Method 3a: Modeling Wood Accumulation

It is possible to model the input, retention, breakdown, and movement of large wood using simulation models (see review of 14 models in Gregory *et al.* 2003b). To illustrate the application of simulation modeling for evaluating ecological and physical processes that influence the storage of wood, we will use a publicly available version of OSU Streamwood (Meleason *et al.* 2003), an integrated model of riparian stand dynamics and wood dynamics in streams of the U.S. Pacific Northwest developed by Mark Meleason.

1. Download OSU Streamwood from the H.J. Andrews LTER website (http://www.fsl.orst.edu/lter/data/tools/models/streamwood.cfm) as a compressed file. Also download the User's Guide to assist in running the model for future applications.

2. Create a folder on your computer's directory named OSU_Streamwood. Unzip the model and place the files in your OSU_Streamwood folder.

3. Double left-click (PC users) to open the StreamWood application file (StreamWood.exe or "StreamWood MFC Application"). Mac users will need to "select" files in all instances.

4. Double left-click on "Environment" under streamwood.bsn in the left window. In this window you can specify the wood dimensions, key wood processes, operation of the riparian stand model, and flow regime.

5. Click the box next to "Use Forest Model" and then click OK.

6. Double left-click on "Sections." OSU Streamwood allows the user to set up a network of stream sections with different riparian conditions or different geomorphic characteristics. The default version has three sections composed of four reaches. For example, S1R1 is "Section 1 Reach 1" and is the downstream reach of the network. S2R1 is immediately above S1R1. S2 indicates that it is the second section and R1 indicates that it flows into section 1. S2R2 is still in section 2 and flows into S2R1 (i.e., longitudinal series of reaches). S3R1 is the third section that flows into section 1. That means that it is a tributary to the mainstem with its confluence at the boundary between sections 1 and 2 of the mainstem (S1R1 and S2R1).

7. Double left-click on each of the reaches. An "environment" tab will appear for each of the four reaches.

8. Double left-click on the "Environment" tab for S1R1. The reach characteristics are described and can be modified. Click on "Same Forest Model Conditions for Both Riparian Zones." Click box next to "Grow a Riparian Forest from 76–100 m from Stream Bank." Note that riparian forest automatically grows from 0–75 m, but this can be modified (unselect) for different forest management regimes. Then click on "Define Riparian Forest Management Regime." This allows you to define the management of both the riparian management zone and the upslope forest. For this phase of the exercise, use the default values and click OK. Note that this will mean that there is no forest harvest in this model run.

9. Repeat this step for the other three reaches.

10. Left click on the "Results" tab on the upper toolbar and then click on "Set Results." This allows the user to set the interval at which the model records the results. The default is a 10-yr interval. Click OK.

11. Left click on the "Run" tab on the upper toolbar and then click on "Model." Type in the name of the simulation. This window allows you to change the time extent for the model run. Change the time from 400 yr to 600 yr. The model is a probabilistic model and the Monte Carlo simulation can be used to explore the variance in model output. For this exercise, do not click "use Monte Carlo" and we will generate a single run of the model.

12. Click "Run."

13. When the hourglass disappears, the model run is complete. You can display the outcomes for each reach. If you click on the down arrow to the right of "Source," you can select the reach to be displayed. If you click on "Variables," you can select the variable to be displayed. The choices include "NuChLog"—number of logs in the active channel, "NuToLog"—number of logs in the active channel, floodplain, and hillslope that touch the channel, "ChanVol"—volume of logs in the active channel, and "Tot_Vol"—volume of logs in the active channel, floodplain, and hillslope that touch the channel. Multiple graphs can be displayed simultaneously by clicking on "Multiline."

14. Click on S2R2. Then click on "ChanVol." Then click on "Multiline." Then click on S2R1 (the downstream reach). The click on S1R1 (the most downstream reach). Note that the wood storage in the channel tends to reach an inflection at approximately 300 yr and the downstream reach continues to accumulate wood because of transport from upstream.

15. You can obtain the numerical values for this model run in the files for Forest and Stream under the Results folder in the OSU_Streamwood folder that you initially created prior to running the model.

E. Advanced Method 3b: Modeling Effects of Timber Harvest on Wood Accumulation

This modification of the previous exercise illustrates the effects of timber harvest on the accumulation of wood in stream channels.

1. Follow steps 1–7 in Advanced Method 3a above.

2. Under the "Environment" tab for S1R1, click on "Same Forest Model Conditions for Both Riparian Zones." Click box next to "Grow a Riparian Forest from 76–100 m from Stream Bank." Then click on "Define Riparian Forest Management Regime." Under the Riparian Management Area box on the left side of the window, set "Years Between Cut" to 50. In this box, "Total RMA Width," "No-Cut Width," "Min Basal Area for Cut," "Min Num of Leave Trees for Cut," and "Min DBH of Leave Trees" should be automatically set at zero. Under the Riparian Forest Outside of RMA box on the right side of the window, set "Years Between Cut" to 50. Then click OK. This simulates a 50-yr harvest rotation with no riparian buffer or management area. Repeat this step for the other three reaches.

3. Left click on the "Run" tab on the upper toolbar and then click on "Model." Type in the name of the simulation. This window allows you to change the time extent for the model run. Change the time from 400 yr to 600 yr. Again, for this exercise, do not click "use Monte Carlo" and we will generate a single run of the model.

4. Click "Run."

5. When the hourglass disappears the model run is complete.
6. In the "Source" box, click on S2R2. Then click on "ChanVol." Then click on "Multiline." Then click on S2R1 (the downstream reach). Then click on S1R1 (the most downstream reach). Note that the wood storage in the channel tends to reach an inflection at approximately 200 yr instead of 300 yr as in the previous nonharvest exercise. Also note the sequence of peaks and declines that reflect the impact of harvest on recruitment of wood to the channel. Lastly, the storage of wood in the channel under a 50-yr harvest cycle was less than 10% of the volume that would accumulate without harvest (or other forms of forest disturbance).

IV. QUESTIONS

1. To what features do you attribute any differences in retention of leaves and wood between the two study reaches? What were the most important retention structures in the two reaches? Were they the same for leaves and wood?
2. Were more leaves retained in pools or in riffles? Why? What are the mechanisms responsible for retention in these two types of bedforms?
3. Did the exponential model adequately describe the POM retention patterns? What exactly do the parameters of this model describe? Are there more appropriate models?
4. What physical features influenced hydraulic retention? Did the measurement of discharge with dye correspond to that determined from the area-velocity technique? What are the limitations of the dye slug release approach?
5. How do you think stream size (order) would affect retention of POM and water? Speculate about retention efficiency in smaller or larger streams than the one you studied. How might discharge and season affect retention in the *same* stream?
6. Compare the wood volumes estimated by direct counts and line-intersect methods. Did they correspond or deviate? Why do you think that is so?
7. In light of your findings, discuss the implications of stream and riparian management practices that tend to reduce the amount of wood loading to streams, to simplify stream channels, or to modify the hydrograph.
8. How would you expect the decay rates of wood (e.g., different tree species, different temperature) to influence the accumulation of wood in a stream reach? How could you use the model to explore this question?
9. How would stream discharge influence the storage of wood in stream reaches? How could you use the model to examine the potential consequences of altered hydrologic patterns on wood dynamics?

V. MATERIALS AND SUPPLIES

Materials for CPOM Releases

Dried or fresh-fallen leaves (e.g., 3000 abscised *Ginkgo biloba* leaves).
Garbage bags (to store leaf batches until released)
Buckets [two 20-L (5-gallon), to soak leaves]
Wood dowels (60 dowels ca. 1 m L × 1.5 cm D)
Fluorescent dye (fluorescein powder or rhodamine-WT liquid)
Current velocity meter (optional)

Dip (D-frame) nets (1 per investigator)
Field notebook with data sheets
Flagging tape
Log calipers (if available)
Meter sticks
Metric tapes (100 m, 50 m, 10 m)
Scintillation vials (100 plastic; numbered)
Seine with lead line (at least as long as the channel width)
Stadia rod and clinometer or hand level (for measuring slope)
Stopwatch
Scintillation vials (to hold dye samples)

Laboratory Equipment for Optional Dye Release

Electronic balance (± 0.01 g)
Fluorometer with filters for specific fluorescent dye
Computer with digitizing software

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Section C

Stream Biota

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Heterotrophic Bacteria

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I. INTRODUCTION

Heterotrophic microorganisms in streams, which include bacteria, protists, and fungi, are important components of the microbial communities associated with the water column; submerged surfaces such as rocks, leaves, and wood; and interstitial water of benthic sediments (e.g., Aumen *et al.* 1983, Bott *et al.* 1984, Stock and Ward 1989, Findlay *et al.* 1993, Lock 1993, Stanley *et al.* 2003). The focus of this chapter is on heterotrophic bacteria, which decompose dissolved (DOM) and particulate organic matter (POM) and are consumed by organisms at higher trophic levels (Meyer 1990).

The field of microbial ecology has undergone extraordinary growth over the last two decades that has resulted in an expanded understanding of the ecological importance of bacteria in stream and other aquatic environments. This growth has been coupled to and driven largely by the development of an array of new techniques that have accelerated our ability to estimate bacterial abundances, growth rates, and productivity in natural and laboratory settings (Zimmerman and Meyer-Reil 1974, Hobbie *et al.* 1977, Porter and Feig 1980, Fuhrman and Azam 1982, Findlay *et al.* 1984, Kirchman *et al.* 1985, Simon and Azam 1989). More recently, the development of highly sensitive biochemical and molecular techniques has enabled progress in understanding bacterial diversity and linkages among microbial composition, spatial arrangement, and function in natural environments (Chrost 1990, Hoppe 1991, Fuhrman *et al.* 1994, Amann *et al.* 1995). With new technology and methods in microbial ecology, we can more thoroughly answer old questions, as well as pose new and exciting ones (Christian and Capone 1997).

One of the continuing challenges in freshwater ecology is to understand the fate of DOM and the roles of microbial communities in determining that fate. In streams, one important role of benthic bacterial communities that has long been recognized is the assimilation of dissolved materials from the overlying water. Both biotic and abiotic retention of DOM (e.g., leachate from leaves) on benthic surfaces is well documented in small stream ecosystems (Cummins *et al.* 1972, Lock and Hynes 1976, Dahm 1981,

McDowell 1985). These processes result in the transfer of organic carbon associated with DOM from the overlying stream water to surfaces, where it can then be partially or wholly metabolized by benthic, heterotrophic microbial communities. Therefore, these communities function to retain and transform DOM, which is an important source of energy. The removal of DOM to benthic habitats by stream microorganisms has primarily been documented by measuring the disappearance of DOM—that is, the decrease in concentration in the overlying water. These studies have revealed that some sources of DOM are removed more rapidly than others, suggesting differences in quality of DOM (Lock and Hynes 1975, Lush and Hynes 1978). *Quality* of organic matter is an imprecise term, but it relates to the ability of organisms to use it as a food source. High-quality DOM should be rapidly assimilated and quickly metabolized by heterotrophic organisms, whereas low-quality DOM should be more slowly utilized (e.g., Hedin 1990).

With the development of methods for estimating bacterial productivity together with an array of other techniques, new approaches to understanding the relationship between microbes and DOM in streams and other environments have evolved. We now know that DOM concentrations are positively correlated with increases in heterotrophic bacterial abundances and productivity in a variety of environments (Findlay *et al.* 1986, Cole *et al.* 1988, Moriarty *et al.* 1990, McKnight *et al.* 1993, Johnson and Ward 1997, Farnell-Jackson and Ward 2003). However, direct measurement of metabolic responses of heterotrophic microorganisms in stream ecosystems to sources of DOM of different quality has been much less studied. We also know little about the role of different sources of DOM in determining the activity and composition of bacterial communities.

Despite the enormous progress that has been made in microbial ecology in recent years, some fundamental characteristics of natural microbial communities in aquatic ecosystems are still not well understood or measured. Over the past decade, several epifluorescent microscopic methods have been developed or fine-tuned for determining bacterial cell activity. Enumeration of acridine orange or DAPI-stained bacterial cells from natural microbial communities yields estimates of total bacterial abundances (Amann *et al.* 1995) but does not distinguish among living, dormant but viable, or dead cells. Active bacterial cells invariably make up a very small percentage (<30%) of total direct counts, which has led to the conclusion that most bacterial particles in aquatic environments are inactive or dead (Beloin *et al.* 1988, Marxsen 1988, Ward and Johnson 1996). The ability to distinguish and quantify active cells will help explain essential ecological attributes such as patterns of microbial growth and productivity and the environmental factors that control these patterns, including responses to DOM (del Giorgio *et al.* 1996, 1997).

In this chapter, two methods are described that are fundamental to understanding key features of heterotrophic bacterial communities: (1) a CTC (5-cyano-2,3-ditolyl tetrazolium chloride) method for identifying and quantifying actively respiring bacteria, and (2) a [³H]leucine method for estimating bacterial productivity. The first method requires a compound microscope equipped with a high magnification (100X), oil immersion lens as well as epifluorescent attachments, including a UV light source and appropriate filter set (described in detail in the following procedures). We have found that the CTC method is more sensitive and less complex than the use of another redox dye and its end-product, INT-formazan, for identifying actively respiring bacteria (Bott and Kaplan 1985, Johnson and Ward 1993). The second advanced method requires use of a weak beta-emitting radioisotope (³H) and appropriate radioassay equipment, including a liquid scintillation counter (Findlay *et al.* 1984, Stock and Ward 1989). These methods estimate metabolic features of bacterial communities by different approaches, but both are applicable to research addressing bacterial response to different quantities and qualities of DOM.

II. GENERAL DESIGN

Use of CTC to Enumerate Actively Respiring Bacterial Cells

The procedures outlined following describe how samples collected from native aquatic habitats can be prepared in the laboratory for microscopic observation of cells stained with the CTC dye. These bacterial cell numbers can be compared to numbers of DAPI stained cells from the same water sample to evaluate the proportion of the total bacterial community that is actively respiring. This procedure involves a 4-hour incubation of the collected water samples with the CTC dye that must occur as soon as possible after the samples are collected. Samples are then killed with formalin; filtered onto black, polycarbonate filters; and prepared for epifluorescent microscopic examination. The following steps can be easily adapted to a number of experimental protocols, including amendment of replicated water samples with known concentrations of different sources of filter-sterilized DOC prior to CTC addition for comparisons with unamended controls in order to determine response of bacterial cell activity to DOC source and concentration.

Estimating Bacterial Production

The tritiated leucine method for estimating bacterial productivity has been broadly used in aquatic ecosystems. The following methods have resulted from a combination of procedures that were adapted for planktonic and benthic communities in stream and wetland habitats. This method provides a sensitive measure of protein production in bacteria that can then be used to estimate bacterial carbon production, the essential unit of production estimates for all organisms. It has also proven to be a valuable measurement of bacterial community response to an array of environmental variables. No specific steps are given below for experimental protocols, but as with the CTC method, the procedures can be easily adapted to compare bacterial responses in water samples amended with DOC to those in unamended samples.

III. SPECIFIC METHODS

A. Basic Method: Estimating Numbers of Respiring Bacteria by Use of CTC Dye

Background

CTC (5-cyano-2,3-ditolyl tetrazolium chloride) methods use a redox probe to show respiratory activity of cells (Rodriguez *et al.* 1992). The CTC redox dye is colorless, but it is reduced by bacterial oxidative respiration to an insoluble, red fluorescing end-product, CTC-formazan, that accumulates intracellularly in bacteria. It is a direct indicator of oxidative metabolism and will not detect dormant or anaerobic cells. CTC is generally similar to INT [2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride], but the bright red fluorescing cells that result from CTC reduction make identification and enumeration of bacteria easier than detection of cells that produce nonfluorescent end-products from INT.

Karner and Fuhrman (1997) compared methods (binding to a fluorescently labeled universal 16S RNA probe; ^{3}H-labeled amino acid uptake via autoradiography; nucleoid-containing bacterial numbers by modified DAPI staining; staining with CTC; and total DAPI counts) to assess bacterial numbers and activity in coastal water communities. They had concerns about CTC techniques because they suspected dissolution of formazan

crystals after CTC reduction may have produced low bacterial counts. However, we have not had this problem with the following method. The ease of identifying brightly fluorescing cells typically results in little error among investigators. In our experiments using the CTC method, we have found that fluorescing cells mounted on filters on slides and stored refrigerated in the dark are stable for at least 24 hours. Also, we have found that numbers of actively respiring bacterial cells identified by the CTC method generally correlate well with bacterial productivity estimated by [³H]leucine techniques (A. K. Ward, personal observation).

General Laboratory Preparation for CTC

1. Prepare CTC working solution from material ordered from a vendor (e.g., Polysciences) either the night before or morning of the assay (see supplies list). CTC stain is packaged in 100-mg quantities in a vial as a dry powder that must be kept refrigerated. To dilute to the proper concentration, add 6.6 mL deionized-water to the vial of dry powder. The powder does not go into solution easily. It can be vortexed several times and, if necessary, sonicated for 5 minutes in a bath sonicator. It is usually necessary to vortex the solution several more times after sonication. Dilute the powder the night before use and then store in a refrigerator (4°C); this allows more time for the CTC to go into solution. When stored in the dark in a refrigerator, the working solution will retain staining effectiveness for approximately 3 weeks.

2. Assemble racks of disposable 15-mL centrifuge tubes, three for each sample. Set the temperature of the growth chamber or incubator at the water temperature anticipated from the field sampling site. Assemble pipets and tips, multiport manifold, and filters, including backing filters (cellulose acetate) and polycarbonate black membrane filters for retaining bacteria for microscopic examination (see supplies list).

Field Sampling

Use either the *planktonic* or *benthic* protocol below, depending on whether you are sampling the water column or a benthic substrate.

1a.   (Planktonic samples) Collect water from the field site and transfer to acid-washed, brown plastic bottles.
1b.   (Benthic samples) Collect the benthic sample (e.g., rock chips, scrapings, portions of macrophytes or leaves, or a measured volume of sediment) and add a known volume of filtered stream water (same method as described for collection of benthic material for bacterial productivity below). Methods for collection of a variety of benthic surfaces and how areas of these surfaces can be quantified are described in detail in Johnson and Ward 1993 and Stanley *et al.* 2003 (see also Chapter 17).
2.   Place sampling containers on ice to transport to laboratory.
3.   Process samples immediately upon return to the laboratory.

Sample Preparation Procedures (Laboratory)

1a.   (Planktonic samples) Pipet triplicate 2.0-mL aliquots from each sample into 15-mL centrifuge tubes.
1b.   (Benthic samples) Sonicate all tubes for 5 min in a bath sonicator to remove bacteria from surfaces. Immediately pipet triplicate 2.0-mL aliquots from each

sample into 15-mL centrifuge tubes. NOTE: The 2.0-mL aliquot represents a proportion of the total bacteria on the benthic surface. That is, if the benthic sample was placed in 10 mL of filtered site water in the field, then the 2.0-mL aliquot contains 20% of the bacteria sonicated from the benthic surface. Therefore, the number of bacteria enumerated from the filter (see below) must be multiplied by a factor of 5 to extrapolate to the entire benthic surface area.

2. Dim room lights. *Important: Sample must be kept in dim light or darkness for the remainder of the procedure.*
3. Pipet 200 μL of CTC solution (diluted from dry material as above) into each tube.
4. Cap tubes and vortex each for 5 seconds to mix CTC with sample.

Sample Incubation (Laboratory)

1. Place test-tube racks with centrifuge tubes onto rotary shaker (e.g., Junior Orbit Shaker, Labline Instruments).
2. Place entire rotary shaker into temperature controlled incubator that is set at the temperature of the aquatic habitat from which the water was collected and adjust to very low speed (i.e., less than 10 rpm).
3. Incubate tubes for 4 hours.
4. Toward the end of the incubation time, set up the 12-port Millipore filtration unit as follows. Place a 0.8 μm pore-size, backing filter (Millipore AAWP filters) on each frit to be used; prewetting the frits with a drop of deionized-water helps insure flat placement of the filter without wrinkles and air bubbles. Place 0.2 μm pore-size, black membrane filters (Osmonics, Inc.) on top of the backing filters (a drop of deionized water also helps here). Tighten the manifold cover and make sure the two red O-rings are properly seated.

Sample Processing (Laboratory)

1. Remove racks from shaker in incubator.
2. Uncap each tube and pipet 53 μL of formalin (37% solution of formaldehyde) into each sample tube (see supplies list).
3. Cap each tube and vortex 5 seconds.

Sample Filtration (Laboratory)

1. Pour entire sample from each tube into a sample well on a Millipore manifold that has previously been fitted with both backing and sample filters (see step 2 under General Preparation above). Decrease the volume filtered, if necessary, to accommodate high bacterial abundances.
2. Turn on vacuum to initiate filtration.
3. When the sample has been filtered, turn off the vacuum and remove the manifold cover.
4. Carefully remove the polycarbonate filter with the bacterial particles from the manifold well with forceps and blot the underside of the filter on Whatman #1 filter paper to absorb any excess moisture.
5. Mount blotted filter on a drop of glycerol on a precleaned, glass microscope slide.
6. Place another drop of glycerol on the top of the filter and gently place a coverslip on top.

Bacterial Quantification (Laboratory)

1. Place a drop of nondrying immersion oil for fluorescence and general microscopy (e.g., Cargille Type DF) on top of the cover slip just prior to microscope examination. Immerse end of appropriate microscope objective (100X) into the oil droplet.
2. For abundance, count the bacteria in at least 10 randomly selected grid fields with an epifluorescence microscope at 1000X total magnification using a UV filter set (450–490 nm excitation range). This is the same filter set used for Acridine Orange stain (e.g., Johnson and Ward 1993). Bacteria will appear bright red against the black background of the polycarbonate filter. Figure 14.1 shows a photomicrograph
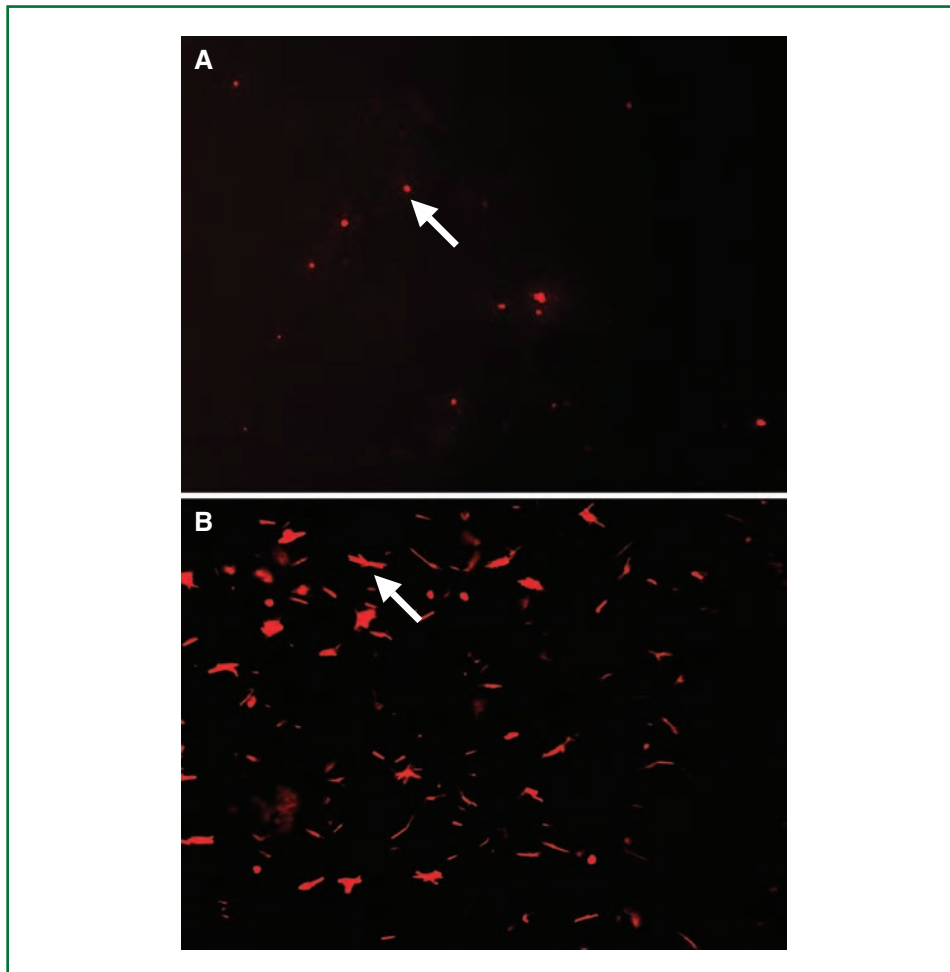
FIGURE 14.1 Bacterial sample from a lotic wetland, Talladega Wetland Ecosystem (Alabama), unamended (A) and after 24 hr of amendment with alder leachate (B). Samples were stained with CTC and viewed using a Zeiss Axioskop microscope with a plan-neofluar 100X oil immersion objective (total magnification 1000X) and Acridine Orange filter set (GI487909) to provide blue light excitation at 450–490 nm. Arrows: Isolated particles in (A) are individual bacterial cells; large particles in (B) are clusters of filamentous bacteria.

of bacteria collected from the Talladega Wetland Ecosystem in Alabama. This wetland was formed by beaver-damming of a small, coastal plain stream and contains ponds connected by a series of meandering streams. The samples illustrated in this figure were collected from a stream segment flowing through alder (*Alnus serrulata*) vegetation. The upper photo (A) shows an unamended stream sample with bacteria stained with CTC, whereas photo (B) shows the same sample of bacteria after alder leachate amendment and stained with CTC. Table 14.1 provides a worksheet to use as a template to calculate the total number of cells per unit area of benthic surface sampled or it can be modified to calculate cells per unit volume of overlying water (plankton).

3. The number of red, fluorescing bacterial cells can be reported as a proportion of total bacterial cells in the sample by dividing abundances of DAPI stained cells (Porter and Feig 1980) into numbers of CTC-fluorescing cells and multiplying by 100. *Caution:* CTC bacterial cells fluoresce in the same red range as autofluorescing chlorophyll-containing particles. Typically, CTC-treated bacterial particles can be easily distinguished from autofluorescing, whole algal cells. However, if amorphous, small fragments of plant material that are in the size range of bacteria are common in water samples used for CTC counts, then a control slide should be included. Control slides include water samples treated as above but without application of the CTC dye. Red, autofluorescing particles in controls should be counted and subtracted from CTC-treated samples.

B. Advanced Method: Assessing Heterotrophic Bacterial Productivity with [³H]leucine Background

The methods for estimating heterotrophic bacterial productivity in aquatic ecosystems have been developed more recently than those for measuring productivity of other organisms such as algae, aquatic vascular plants, and macro-animals. Early methods focused on the measurement of nucleotide ([³H]thymidine) incorporation into bacterial DNA during DNA synthesis as an estimate of the rate of bacterial biomass production (e.g., Fuhrman and Azam 1982). These techniques were initially developed for ocean/estuarine plankton systems, but have been adapted to lotic ecosystems, including benthic habitats (Findlay *et al.* 1984, Stock and Ward 1989, Hudson *et al.* 1990, Kaplan *et al.* 1992). More recently, the use of amino acid ([³H]leucine) incorporation into bacterial protein has also gained acceptance as an estimate of bacterial productivity (Kirchman *et al.* 1985, Wetzel and Likens 1991). [³H]leucine was chosen over other amino acids because the leucine content of bacterial protein remained more constant than other amino acids. The results from the bacterial protein production (BPP) method have shown good correspondence with results from [³H]thymidine studies (Kirchman and Hoch 1988), and the technique is a more direct and less complicated procedure than the [³H]thymidine method for estimating bacterial productivity.

The development of a technique for estimating heterotrophic bacterial productivity has been valuable to the field of aquatic ecology because we now have a measurement for bacteria that allows comparisons across aquatic systems and with productivity measurements for organisms in other trophic levels (i.e., C per volume or area per time). Therefore, both the quantitative importance of bacterial productivity in aquatic ecosystems and the roles of bacteria in microbial food webs and ecosystems can now be more thoroughly evaluated (e.g., Findlay *et al.* 1986, Simon and Azam 1989, Stanley *et al.* 2003). Despite much progress in this area in recent years, radioisotope methods for estimating bacterial

TABLE 14.1 Worksheet for Determining Abundances of CTC-fluorescing Cells per Unit Area of Benthic Surface (See Text for Description of Preparation Procedures). Code for hypothetical samples: R = rock sample followed by replicate number and L = leaf sample followed by replicate number; "Volume sonicated" refers to volume of water (e.g., 10 mL) in which the benthic sample was placed when sonicated; "Volume incubated" is the subsample of volume sonicated that was incubated with the CTC (e.g., 2 mL); "Multiplier A" is the factor calculated by dividing 100 by the percentage volume incubated (e.g., given volumes above, 100% divided by 20% = 5); "Multiplier B" is the factor calculated by dividing 100 by the percentage area of the filter counted in order to extrapolate cell counts to total on filter; "# cells counted $\times A \times B$ divided by benthic area in cm^{2}" provides the number of CTC cells per cm^2 of benthic surface.

CTC Worksheet for Benthic Bacterial Abundance[1]

Date of sample collection: _____

Investigators: _____

SITE	SAMPLE	Volume Sonicated (mL)	Volume Incubated (mL)	Percentage Incubated	Multiplier A (100 ÷ % incubated)	Percentage filter area counted	Multiplier B (100 ÷ % filter counted)	# cells counted on filter	# cells $\times A \times B$ divided by benthic area in cm^2 (CTC cells/cm^2)
A	R-1								
	R-2								
	R-3								
	L-1								
	L-2								
	L-3								

[1] *This worksheet can be set up in Excel format with relevant calculations entered for columns progressing from left to right. The worksheet above applies to benthic samples, but it can be easily modified for planktonic samples.*

productivity in aquatic ecosystems are not without technical and interpretation problems. Recent reviews (e.g., Robarts and Zohary 1993) and journal articles (e.g., Jørgensen 1992a, b, Marxsen 1996, Buesing and Gessner 2003, Buesing and Marxsen 2005) discuss methods and/or problems associated with nonspecific labeling, isotope dilution, and calculation of conversion factors to arrive at units of growth or productivity.

In this exercise, a method for measuring [³H]leucine incorporation into bacterial protein is outlined for estimating bacterial productivity. These procedures were adapted from the literature cited above and from isotope dilution experiments in planktonic and benthic, freshwater habitats (Thomaz and Wetzel 1995, Ward unpublished data). Methods are described for both planktonic and benthic habitats. For each habitat, triplicate killed controls and triplicate samples are used. Samples are collected, transferred to large test tubes, and incubated *in situ* for short time intervals (e.g., 30 min). The incubation is terminated by adding formalin, and samples are taken back to the laboratory for further processing.

Treatments consisting of DOC amendments and temperature can easily be incorporated into the exercise. DOC amendments should be made to samples and killed controls prior to addition of the [³H]leucine. Preincubation with DOC can vary from one-half hour to several hours or longer before the addition of [³H]leucine. Temperature effects can be evaluated by transporting samples to the laboratory before addition of the [³H]leucine and incubating samples in temperature controlled waterbaths or growth chambers.

The amount of leucine added in this type of experiment must saturate bacterial uptake kinetics without causing increased metabolism because of increased nutrient availability. Freshwater, planktonic bacterial uptake of leucine typically saturates at a concentration of about 100 nM (Jørgensen 1992a, b), although saturation values from stream sediment samples have been reported at much higher levels in the 10–50 µM range (Marxsen 1996, Buesing and Marxsen 2005). In order to conserve expensive radiolabeled leucine, a mixture of both radiolabeled and nonradiolabeled leucine is used to accomplish this saturation. Sediments and epilithic microhabitats typically have higher saturation requirements than planktonic habitats. We suggest a leucine concentration of 300 nM in the benthic habitat protocol below. The actual concentrations needed to saturate leucine uptake can vary markedly from one system to another and can be determined by methods described in Jørgensen (1992a, b) and Thomaz and Wetzel (1995). This procedure is relatively straight forward and is highly recommended in order to ensure the most accurate estimates of bacterial carbon production.

General Preparation for Production Laboratory

1. Assemble and label necessary supplies (see supplies list; Section V). Autoclave test tubes, volumetric flasks to dilute radiolabeled and non-radiolabeled leucine, and pipet tips.
2. Arrange field supplies in an appropriate field kit (see Section V).

Production Procedure (Laboratory Preparation)[1]

1a. (Planktonic samples) Make 10 µM leucine (nonradioactive) solution by dissolving 0.0013 grams of L-leucine in 1 liter of sterile, deionized (DI), high purity water in a volumetric flask.

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[1]Use sterile procedures as much as possible (in laminar flow hood, if available).

1b. (Benthic samples) Make 30 μM leucine (non-radioactive) solution by dissolving 0.0039 grams of L-leucine in 1 liter of sterile, DI, high-purity water in a volumetric flask.

2. Make [^{3}H]leucine solution by diluting stock solution from manufacturer so that 50 μL of the final, diluted, radiolabeled solution added to a 10-mL sample yields a 10 nM [^{3}H]leucine solution. For example, add 1.0 mL of a [^{3}H]leucine solution of specific activity of 143 Ci/mmol (5 mCi/mL) to 16.5 mL of sterile DI water of high purity.

3. Add 2.5 mL of the [^{3}H]leucine solution from step 2 to 5.0 mL of the unlabeled leucine solution from step 1. Transfer to a sterile, capped container that can be transported to the field site in the field kit.

Production Procedure (Field)

Option: If temperature effects are to be investigated, collect samples and transport immediately to the laboratory for the following procedures:

1. Pipet 0.4 mL of 100% formalin into killed control tubes.

2a. (Planktonic samples) Pipet 10.0 mL of unfiltered site water into all test tubes (killed controls and samples) with screw tops.

2b. (Benthic samples) Pipet 10.0 mL of filter-sterilized site water into all test tubes (killed controls and samples) with screw tops, then add benthic sample to the tubes, (e.g., rock chips, portions of macrophytes or leaves, or a measured volume of sediment), and attach screw tops to tubes.

3. Equilibrate all test tubes at *in situ* temperature for 30 min. This procedure ensures that there is sufficient time for the bacteria in the killed controls to be affected adequately by the formalin. Also if there are any changes in temperature of the water caused by transferring it to the test tubes, then there is time for the temperature of the water to be restored to the temperature of the native habitat before the addition of the leucine.

4. Unscrew tops.

5. Pipet 150 μL radiolabeled/non-radiolabeled leucine mix solution to all tubes. Record time accurately (with a digital watch) on data form.[2] It requires about 5 seconds to pipet the leucine solution into each tube.

6. Replace tops and swirl tubes gently to mix (or vortex in laboratory); place back into sampling habitat or in laboratory incubator for 30 min.

7. At end of incubation, add 0.4 mL of 100% formalin to each sample tube and mix. Record time accurately on form. Transport to laboratory as soon as possible.

Production Procedure (Laboratory)

1. (Benthic samples only) Sonicate all tubes for 5 min in a bath sonicator.

2. Add 3.25 mL of 15% TCA (trichloroacetic acid) to all tubes and vortex carefully. TCA is a common reagent used to precipitate cellular protein.

3. Place tubes in a 95°C water bath for 30 min. This process augments the protein precipitation process.

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[2] One investigator can monitor a digital watch, indicate when to pipet, and note the time on the data sheet while another person pipets.

4. Remove from water bath and cool at room temperature for approximately 20 min.
5a. (Planktonic samples) Filter the whole sample onto a 25-mm-diameter, polycarbonate filter of 0.2 μm pore size. Use a filtration pressure of no more than 150 mm Hg.
5b. (Benthic samples) Filter using appropriate aliquots depending upon sample type (e.g., 0.5 mL for sediments).
6. After filtration of the sample, but while the filter is still on the filtration frit, rinse each filter at least seven times with 5-mL aliquots of DI water to remove as much abiotically adsorbed radiolabel as possible. We have found that this treatment reduces radiolabel adsorbed to a polycarbonate filter to background levels. Alternatively, filters can be rinsed with 2 mL of cold 80% ethanol (Wetzel and Likens 2000).
7. Remove each filter from the filtration apparatus and place in a labeled scintillation vial with 10 mL of an appropriate scintillation cocktail (e.g., AquaSol®-2). Samples should be radioassayed by liquid scintillation to yield DPM/vial.

Production Calculations

1. Subtract average DPM of killed control tubes from average DPM of sample tubes.
2a. Use the following formula to calculate mol leucine$_{inc}$ L^{-1} h^{-1} for planktonic samples:

$$\frac{(dpm_{sample} - dpm_{killed})(mL/liter)(min/h)(formalin\ addition\ factor,\ 1.03)}{(dpm/Ci)(specific\ activity\ in\ Ci/mmol)(vol\ filtered,\ mL)(incubation\ time,\ in\ min)(mmol/mol)},$$

$$(14.1)$$

where dpm/Ci $= 2.2 \times 10^{12}$, and mmol/mol $= 1000$.
2b. Use the following formula to calculate mol leucine$_{inc}$ cm^{-1} hr^{-1} for benthic and surface samples:

$$\frac{(dpm_{sample} - dpm_{killed})(min/h)(sample + TCA,\ 13.25\ mL)(formalin\ factor,\ 1.03)}{(dpm/Ci)(specific\ activity\ in\ Ci/mmol)(surface,\ cm^2)(incubation\ time,\ in\ min)(mmol/mol)(aliquot)}$$

$$(14.2)$$

where aliquot = mL subsample filtered of 13.25 mL in the incubation tube.
3. In addition to having DPM measurements, the other factors used in the equations below need to be considered. Determine BPP by multiplying the moles of exogenous leucine incorporated by 100/mol% of leucine in protein (7.3), and by the gram molecular weight of leucine (131.2). This value is multiplied by an intracellular isotope dilution (ID) of leucine if one is known. A typical ID factor of 2 (Wetzel and Likens 2000) is based on an intracellular isotope dilution of 50%, determined from studies of marine bacterioplankton (Simon and Azam 1989). However, Jørgensen (1992a) concluded that freshwater bacteria have more variable isotope dilutions than marine bacteria, ranging from 30 to 90%, which would

result in ID factors of 3.3 to 1.1. We include a factor of 2 in the calculations below as representative of a reasonable middle value. External dilution (ED) is entered into the calculations to account for the ratio of labeled to unlabeled leucine. ED is comprised of two parts: the dilution caused by the ambient amount of leucine in the water, and the dilution caused by the amount of unlabeled leucine added with the [^{3}H]leucine. In the planktonic procedure described above a 10 nM [^{3}H]leucine concentration and a 100 nM leucine concentration are added to ambient concentrations in the stream water, resulting in a dilution factor of 11. In the benthic procedure described above, a 10 nM [^{3}H]leucine concentration and a 300 nM leucine concentration are added to ambient concentrations in the stream water, resulting in a dilution factor of 31. The amount of leucine normally found in freshwater systems is quite low, normally less than 5 nM and often less than 1 nM. If the ambient concentration is known, this factor can also be added. We do not include the naturally-occurring, ambient, leucine ED in the calculations below.[3] The complete formula is:

$$BPP(g) = (mol\ leucine_{inc})\ (100/mol\%leucine)\ (leucine\ mw)\ (ID)\ (ED), \quad (14.3)$$

or simplified,

$$BPP(g) = (mol\ leucine_{inc})\ (100/7.3)\ (131.2)\ (ID)\ (ED), \quad (14.4)$$

or further simplified,

$$BPP(g) = (mol\ leucine_{inc})\ (1797)\ (2)\ (ED). \quad (14.5)$$

4. Convert BPP to bacterial carbon production by multiplying BPP by 0.86.
5. The resulting value is bacterial carbon production per unit volume or area per hour. A worksheet form that can be used for calculating bacterial carbon production is provided in Table 14.2.

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[3] The formulas here are derived from Simon and Azam (1989). See that reference for further details and rationale for the method.

TABLE 14.2	Worksheet for Determining Bacterial Productivity per Unit Area of Benthic Surface Using Tritiated Leucine Method. Code for abbreviations in table: S = sample followed by replicate number; K = killed control followed by replicate number; hh = hour, mm = minute, ss = second; INCUB = incubation; DPM = disintegrations per minute; DPM BLANK refers to the background DPM as recorded by the liquid scintillation counter from a vial with scintillation cocktail and dissolved filter, but no radiolabel; LEUC INCOR = leucine incorporated; BPP = bacterial protein production; BCP = bacterial carbon production; BCP mean = average value for 3 replicates; BCP se = standard error for 3 replicates.

Tritiated Leucine Worksheet for Benthic Bacterial Productivity[1]

Specific activity of tritiated leucine (Ci/mmol):_____

Date of sample collection:_____

Investigators:_____

SITE	SAMPLE	START TIME (hh:mm:ss)	KILL TIME (hh:mm:ss)	INCUB TIME (min)	DPM	DPM BLANK	LEUC INCOR $(g/cm^2/hr)$	BPP $(g/cm^2/hr)$	BCP $(gC/cm^2/hr)$	BCP mean $(gC/cm^2/hr)$	BCP se $(gC/cm^2/hr)$
A	S-1										
	S-2										
	S-3										
	K-1										
	K-2										
	K-3										

[1] *This worksheet can be set up in Excel format with relevant calculations entered for columns progressing from left to right (see text for appropriate equations and factors used in the calculations). The worksheet above applies to benthic samples, but it can be easily modified for planktonic samples by substituting equations suitable for volume (e.g., gC/L/hr) rather than area (e.g., gC/cm² /hr) in calculations for BPP and BCP (see text).*

IV. QUESTIONS

Estimating Numbers of Respiring Bacteria

1. Why are such small percentages of total bacteria in most aquatic environments actively metabolizing (respiring)?
2. What kinds of environmental stimulants would likely result in increased numbers of actively respiring cells?
3. Would higher numbers and percentages of active cells likely occur associated with microbial communities attached to surfaces in streams or in the overlying water? Why?
4. What is the ecological significance of the occurrence of large numbers of dormant, yet viable, bacterial cells of different taxa and function in stream and other aquatic habitats? That is, would this be an advantage, disadvantage, or neither to microbial community and ecosystem function? Why or why not?

Bacterial Production

5. Typically, starved bacteria divide repeatedly without growing. What effect would this have on interpreting results from the [^{3}H]leucine incorporation into protein method and the [^{3}H]thymidine incorporation into DNA?
6. In assessing overall ecological importance of bacteria, what are the advantages of knowing rates of bacterial production in addition to abundance and/or biomass?
7. Some anaerobic bacteria do not take up appreciable amounts of dissolved thymidine or leucine. If your sample substrata contain anaerobic microzones, how would this affect your interpretation of the results?
8. In the experiments described in this exercise, leucine concentrations are increased to a value that theoretically is in the saturating range for leucine uptake by native bacterial communities. The assumption is that measurable, natural leucine concentrations are low (e.g., 5 nM or less). How would substantially higher, natural concentrations of leucine affect the calculations?
9. Would you expect that heterotrophic bacterial productivity would always be positively correlated with bacterial abundances and/or biomass? Why or why not?

V. MATERIALS AND SUPPLIES

Estimating numbers of respiring bacteria by use of CTC

> 12-port sampling manifold (e.g., Millipore Inc.)
> 15-mL disposable, sterile centrifuge tubes
> Black polycarbonate filters, 0.2-μm pore size
> Cellulose acetate filters, 0.8-μm pore size

Compound microscope equipped with: (1) oil immersion lens ≥1000X total magnification; (2) UV light source and filter set; and (3) ocular counting grid and measuring reticle. The filter set used for viewing CTC stained cells is the same as is used for viewing Acridine Orange stained cells. The individual filters are: (1) Excitation 450–490; (2) Dichroic mirror FT 510; and (3) Emission LP 520.

Coverslips
CTC stain (Cyanoditolytetrazolium chloride) (Polysciences, Inc.)
Deionized (e.g., Milli-Q) water
Forceps
Formalin
Glycerol
Immersion oil, Cargille type DF
Microscope slides
Pipets
Vacuum pump

Heterotrophic bacterial production

^{3}H-leucine ($\sim$150 Ci/mmol)
0.2-μm polycarbonate filters
15% Trichloroacetic acid (TCA)
AquaSol®-2 scintillation cocktail
Auto-pipetter(s) and tips
Disposable 0.2-μm filter unit (field)
Filter tower(s) or manifold
Formalin
Hand pump (field)
Large (65-mL) screw-top test tubes
Liquid scintillation counter
Scintillation vials
Sonicator
Test tube rack(s)
Vacuum pump
Vortex
Water bath and thermometer

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Fungi: Biomass, Production, and Sporulation of Aquatic Hyphomycetes

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I. INTRODUCTION

Fungi are common inhabitants of stream ecosystems. All phyla of true fungi (Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota) and also the Oomycota (kingdom Stramenopila) that are morphologically similar to fungi can be observed in or isolated from stream environments. The most ecologically important and well studied fungi in streams are the "aquatic hyphomycetes," which are anamorphs (asexual stages) of ascomycetes or basidiomycetes. These fungi are capable of completing their entire asexual life cycle underwater starting from colonization of suitable substrate followed by intramatrical mycelial growth and abundant sporulation. Up to 80% of fungal production may be invested into conidia (i.e., asexual spores) (Suberkropp 1991). Conidia of aquatic hyphomycetes are often tetraradiate, variously branched, or filiform (rarely conventionally shaped) (Figure 15.1), which is an adaptation to dispersal in flowing water and enhances the probability of attachment to fresh substrates (Webster and Descals 1981).

Allochthonous organic matter (leaves of deciduous trees, twigs, etc.) is an important source of energy and nutrients in small forest streams (Fisher and Kaushik 1968, Likens and Hynes 1973) and fungi are the main colonizers of this plant litter. Maximum fungal biomass and sporulation rate of aquatic hyphomycetes correlate well with plant litter breakdown rate (Gessner and Chauvet 1994, Niyogi *et al.* 2003), suggesting that fungi are
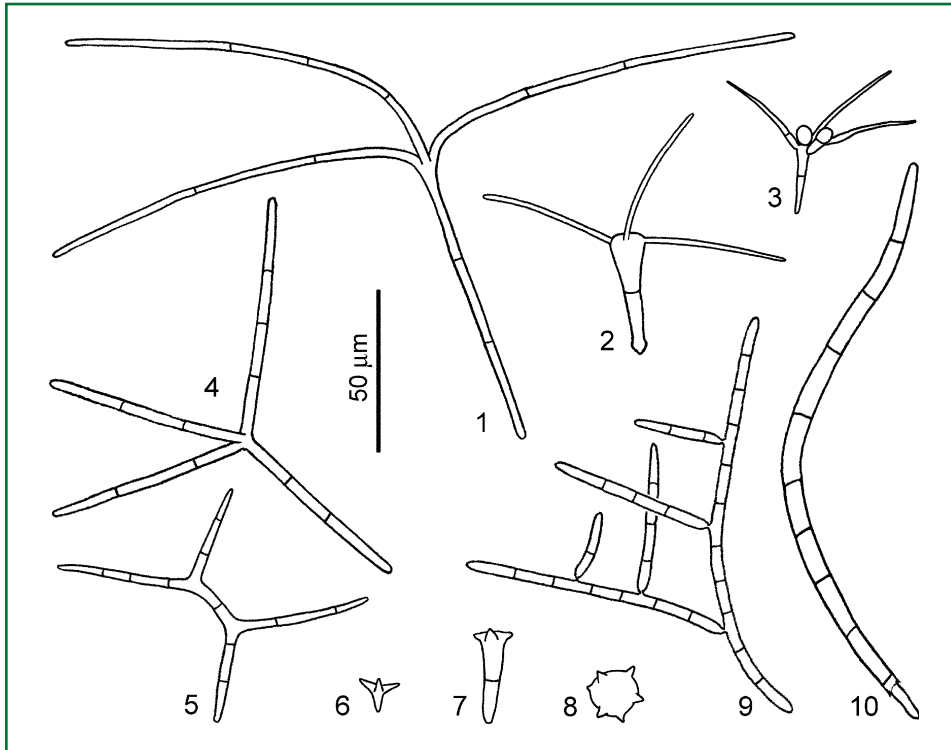
FIGURE 15.1 Conidia of aquatic hyphomycetes. 1. *Tetrachaetum elegans*. 2. *Clavariopsis aquatica*. 3. *Tetracladium marchalianum*. 4. *Lemonniera aquatica*. 5. *Tricladium angulatum*. 6. *Heliscella stellata*. 7. *Heliscus lugdunensis*. 8. *Goniopila monticola* (or *Margaritispora aquatica*). 9. *Varicosporium elodeae*. 10. *Anguillospora longissima*.

the primary decomposers of this organic matter in headwater streams. Fungal biomass accounts for 95 to >99% of total microbial biomass (fungi plus bacteria) associated with submerged decaying plant litter (Baldy and Gessner 1997, Hieber and Gessner 2002, Gulis and Suberkropp 2003a) and fungal production is also typically greater than that of bacteria (Weyers and Suberkropp 1996, Baldy *et al.* 2002). Annual fungal production on an areal basis in small streams can be comparable to or higher than invertebrate secondary production (Suberkropp 1997, Methvin and Suberkropp 2003). Fungi mineralize organic carbon of plant litter, convert it into their own biomass, which can account for up to 18–23% of total detrital mass (Suberkropp 1995, Methvin and Suberkropp 2003), macerate leaf litter, and release fine particulate organic matter (including conidia) and dissolved organic matter that become available to other stream dwellers. Fungal colonization results in "conditioning" of leaf litter that increases its nutritional value and palatability to shredding invertebrates. It is well documented that aquatic invertebrates prefer to feed and grow better on leaf litter colonized by fungi in comparison to uncolonized leaves (Bärlocher 1985, Suberkropp 2003). Therefore, apart from the decomposition of organic matter, aquatic hyphomycetes also mediate energy and nutrient transfer to higher trophic levels (Bärlocher and Kendrick 1976).

   Despite their key importance in the functioning of stream ecosystems, aquatic fungi, historically, have received much less attention than macroinvertebrates, algae, fishes, or

even bacteria. Aquatic hyphomycetes were discovered as a distinctive ecological group only in the middle of the last century (Ingold 1942). This may be explained in part by the microscopic size and habitat of these organisms (inside opaque submerged decaying substrates). Stream ecologists recognized the importance of fungi about three decades ago (Kaushik and Hynes 1968, Bärlocher and Kendrick 1974, Suberkropp and Klug 1976). Several useful reviews summarizing achievements in the field have been published since then (Suberkropp 1992, Bärlocher 1992, Gessner *et al.* 1997, Gessner and Newell 2002, Gessner *et al.* 2003).

This chapter focuses on quantitative methods to study the ecology of aquatic fungi. Specific objectives are to (1) describe techniques to determine concentration of conidia and community structure of aquatic hyphomycetes in transport; (2) present approaches for estimating reproduction (sporulation rate) of aquatic hyphomycetes; and (3) introduce methods to estimate fungal biomass and production associated with decaying submerged plant litter.

II. GENERAL DESIGN

A. Site Selection and General Considerations

Submerged decaying plant litter that serves as substrate for aquatic fungi can be found in almost all types of lotic habitats. Alternatively, plant material introduced as leaf bags or packs can be used after appropriate stream exposure. Collected leaf litter, woody substrates, dead macrophytes, or other organic materials are suitable for determination of fungal biomass and production since these assays are designed to target a broad group of fungi of mainly ascomycetous and basidiomycetous affinities (Gessner and Newell 2002). If the objectives of the study also include estimation of sporulation rate and/or community structure of aquatic hyphomycetes, then headwater streams may be the best choice because of the greater abundance and diversity of these fungi in fast flowing well-aerated streams. Aquatic hyphomycetes can also be found in large rivers (e.g., seventh order; Baldy *et al.* 2002) and specific objectives of the study (e.g., effect of pollution, inorganic nutrients, pesticides, etc. on these fungi) may dictate the choice of site.

B. Sampling Conidia of Aquatic Hyphomycetes from the Water Column

Iqbal and Webster (1973) proposed a straightforward technique to study conidia of aquatic hyphomycetes in transport based on filtering a known volume of water through a membrane filter, staining trapped conidia, and subsequent microscopic examination to count and identify spores. Conidia of aquatic hyphomycetes can often be identified to species due to their characteristic shapes (Ingold 1975, Webster and Descals 1981, Marvanová 1997, Gulis *et al.* 2004). A slightly modified method is described here based on our own experience.

Concentrations of aquatic hyphomycete conidia in stream water can vary over a wide range (100 to 25,000 per liter) (e.g., Suberkropp 1991, Gulis and Suberkropp 2005). The amount of seston carried by the stream also varies considerably over space and time (e.g., Lamberti and Resh 1987). Preliminary trials in a chosen stream may be useful to estimate the volume of water to be filtered in order to trap enough conidia on the filter while preventing interference from too much debris when examining the filter with the microscope. In most streams, 0.2–1.0 liter is a reasonable compromise.

C. Fungi Associated with Plant Litter

Although sporulation rate, fungal biomass and production can be estimated from different types of decaying plant litter, we use here leaf litter in all experimental protocols. Since microbial parameters should be standardized by ash-free dry mass (AFDM) of plant litter, two identical sets of subsamples for biomass and production experiments should be taken (or three sets total if sporulation, biomass, and production are estimated at the same time). Sets of disks can be easily cut from leaves, one set is used to determine AFDM and the other (or other two) for analyses.

D. Sporulation Rate of Aquatic Hyphomycetes

We describe the method to induce sporulation from substrates collected in the field using specially designed laboratory incubation chambers (Figure 15.2) proven to adequately simulate stream conditions with respect to turbulence and aeration necessary to trigger conidia production in many species of aquatic hyphomycetes. The chambers can be ordered from a local glass blower. The alternative is to use Erlenmeyer flasks aerated aseptically through glass tubes or pipettes (Bärlocher 1982, Maharning and Bärlocher 1996) or agitated on a shaker (Baldy *et al.* 1995, Hieber and Gessner 2002). However, the resulting
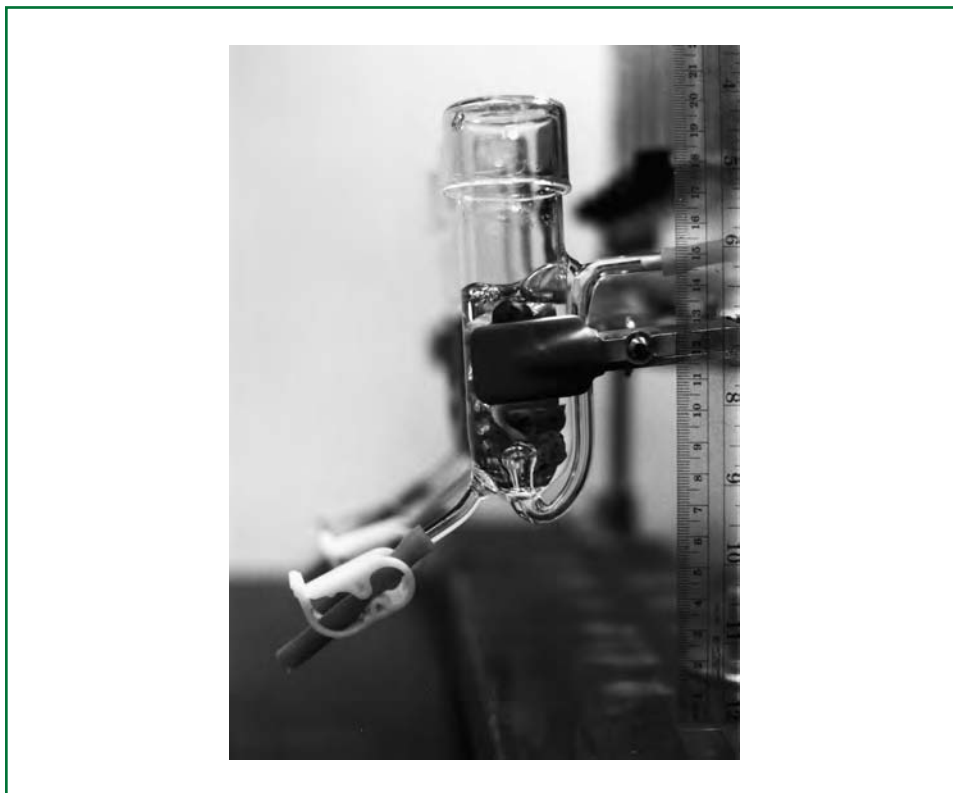
FIGURE 15.2 Laboratory microcosm used to induce sporulation of aquatic hyphomycetes (after Suberkropp 1991).

conidia production estimates from different methods have not been directly compared and may differ somewhat. We strongly recommend filtering conidia suspensions and preparing microscopic slides immediately when laboratory incubations are terminated. However, if a large number of samples are to be processed, suspensions can be fixed with formalin (2% final concentration) in plastic centrifuge tubes and processed later (Hieber and Gessner 2002). It is not known what losses in conidia such a procedure may cause.

E. Fungal Biomass

Determination of fungal biomass by direct microscopic biovolume estimates has been proven to be unsatisfactory, since fungal mycelia grow inside opaque plant tissues and problems with separating mycelium from leaf tissue or clearing leaf tissue result in severe underestimates (Gessner and Newell 2002). ATP and chitin assays have been proposed to quantify fungal biomass, but, because of complexity of analysis, lack of selectivity, and/or other pitfalls, they will not be considered here. The method adapted for this chapter is based on determination of the specific biochemical marker, *ergosterol*, which is the major membrane sterol in higher fungi (Gessner and Newell 2002). Ergosterol is thought to be a measure of living biomass since it is prone to fast degradation upon cell death and membrane disruption.

Liquid-to-liquid extraction of ergosterol from plant litter samples (Newell *et al.* 1988 as modified by Suberkropp and Weyers 1996) is a commonly used technique. Caution should be exercised to protect samples from sunlight as UV degradation of ergosterol will occur. Ergosterol is first extracted and saponified by refluxing plant material in alcoholic KOH. The lipid fraction is partitioned into a non-polar solvent and evaporated to dryness. Ergosterol is redissolved in methanol, filtered, and further separated and quantified by high performance liquid chromatography (HPLC). A second method to extract ergosterol after refluxing and saponification is a solid phase extraction (SPE) using reverse phase extraction columns (Gessner and Schmitt 1996). This method allows more samples to be processed in a given time and has been proven reliable in laboratories with high sample throughput and appropriately trained personnel. However, we do not describe this method here because the exact protocol can vary depending on column manufacturer and it involves acidification of extracts that can cause degradation of ergosterol (Gessner and Newell 2002).

F. Fungal Production

Newell and Fallon (1991) introduced the only method available for determining instantaneous growth rates and biomass production for litter decomposing fungi. This method has been modified for use with stream fungi (Suberkropp and Weyers 1996, Gessner and Chauvet 1997, Suberkropp and Gessner 2005) and involves determining the rate of incorporation of radiolabelled acetate into ergosterol. Fungal growth rates are proportional to rates of acetate incorporation into ergosterol and can be calculated using empirical or theoretical conversion factors (Gessner and Newell 2002). Fungal production is then calculated by multiplying growth rates by biomass (determined from ergosterol concentrations).

Leaf disks may be taken from leaves contained in litter bags to determine production during decomposition (Weyers and Suberkropp 1996) or from naturally occurring leaves to determine fungal production for an entire stream reach (Suberkropp 1997). Leaf disks

are incubated in stream water to which radiolabelled acetate is added. Samples are aerated during incubation for 2–5 h depending on the level of activity. Leaf disks are placed in methanol and later ergosterol is extracted and separated with HPLC. The ergosterol fraction is collected and its radioactivity determined with a scintillation counter. All steps are carried out using proper procedures and precautions for handling radioactive samples and waste.

III. SPECIFIC METHODS

A. Basic Method 1: Conidia of Aquatic Hyphomycetes in Water Column

Field Protocol

1. Take a water sample of 0.2–1.0 liter directly with a graduated cylinder if possible or subsample a larger grab sample. Care should be taken to not disturb benthic sediments. It is preferable to sample riffles since there are indications that shallow calm pools can serve as sinks for conidia. Filter the water sample immediately through a mixed cellulose ester membrane filter (47 mm diam., 5-8 μm pore size) using a hand pump and applying a gentle vacuum (less than 20 cm Hg) to avoid distortion of aquatic hyphomycete conidia.
2. Release the vacuum and cover the filter with a solution of trypan blue (or cotton blue) in lactic acid with a dropper. Pull the excess stain through the filter with vacuum and transfer the filter into a Petri dish with a tight-fitting lid, conidia side up. Filters should stay moist and can be stored for several months.
3. Rinse the cylinder and filter holder with stream water between samples.

Laboratory Protocol

1. Cut the filter in half, mount each half on a microscopic slide with 2–3 drops of the trypan blue (or cotton blue) stain, and add a 22 × 40 mm coverslip. Avoid lateral movement of the coverslip since this may distort conidia.
2. Scan filters with a compound microscope at 150–200× magnification to count and identify conidia of aquatic hyphomycetes. Record the number of microscopic fields counted per filter.
3. Use Table 15.1 to enter data. We recommend scanning at least 150 microscopic fields (area *ca.* 1 mm^2 each) per filter or until at least 200 conidia are counted.

Data Analysis

1. Aquatic hyphomycete conidia concentration (C) in water (no./L) can be calculated as:

$$C = \frac{n \cdot A_e}{f \cdot A_{mf} \cdot V}$$

(15.1)

TABLE 15.1	Sample Data Sheet for Enumeration of Aquatic Hyphomycete Conidia on Filters (some data are filled in as guidance)		

Stream_____   Date_____   Filter ID_____
Volume filtered (L) <u>0.5</u>
Effective filtered area (mm^2) <u>1018</u>   Microscopic field area (mm^2) <u>0.92</u>

Aquatic hyphomycete	Conidia counted		
	Half A	Half B	A+B
Alatospora acuminata	15	13	28
Anguillospora longissima	25	19	44
. . . . etc.	. . . . . .	. . . . . .	. . . . . .
Total conidia counted	105	98	203
Number of species	16	15	19
Fields counted	75	75	150

where n is the number of conidia counted; A_e, effective filtered area (determined from the inside diameter of the filter holder in mm^2); f, number of fields counted; A_{mf}, microscopic field area (mm^2); V, volume of water filtered (L).

2. To analyze community structure of aquatic hyphomycetes or to make comparisons between streams, traditional metrics such as diversity, evenness, and similarity can be calculated based on relative abundances of conidia of individual species. To account for differences in sample sizes when comparing species richness of aquatic hyphomycetes, rarefaction as commonly used for stream invertebrates (i.e., based on number of individuals collected) can be used. The alternative is to standardize on the volume of water from which conidia were counted rather than on the number of individuals (conidia) counted (see Bärlocher and Graça 2002, Gulis and Suberkropp 2004).

B. Basic Method 2: Sporulation Rate of Aquatic Hyphomycetes

Field Protocol

1. Take a water sample (acid-washed plastic bottle) sufficient to have 40 mL for each laboratory microcosm used to induce sporulation.
2. Collect submerged decaying leaves, rinse them in the stream, and either transport to the laboratory in stream water (or Ziploc® bags) in a cooler or process in the field as follows.
3. For each sample, cut 10 leaf disks with an 11.2 mm diameter cork borer avoiding main veins. Place disks in a wide-mouth jar filled with stream water and transport back to the laboratory in a cooler.

Laboratory Protocol

1. Filter stream water (Whatman GF/F) and use 40 mL to fill each incubation chamber (Figure 15.2).
2. Place a set of 10 disks in each sporulation chamber, adjust air flow to 80–100 mL/min, and incubate at 15°C (or prevailing stream temperature) for

24±2h. Rather strict standardization of incubation time is necessary since sporulation rate through time may be nonlinear.

3. Stop aeration and record incubation time (±5 min). Slowly drain the conidia suspension into a 100-mL beaker rinsing the inner walls of the chamber with the suspension (5-mL pipettor) to remove attached conidia.

4. Add 100 μL of Triton X-100 solution and a stirring bar to each beaker and stir gently for several minutes (150–200 rpm) to achieve uniform distribution of conidia.

5. Take a 2–20 mL aliquot (or make dilutions if necessary), transfer onto 25 mm diameter, 5–8 μm pore size membrane filter and apply gentle vacuum. Rinse the walls of the funnel with distilled water so all conidia are collected and prepare the filter as described in Basic Method 1. Mount the entire filter on a microscopic slide applying a drop of stain on top of the filter and add a 22×22 mm coverslip.

6. Transfer leaf disks from each incubation chamber into preashed and preweighed small crucible or aluminum weighing boat and dry to a constant weight at 100°C (e.g., 2 d). Let cool in a desiccator for 10 min and weigh to the nearest 0.1 mg. Combust at 500°C for at least 4 h or overnight, reweigh and calculate AFDM by difference (see also Chapter 17).

7. All prepared filters should be checked under the microscope (*ca.* 15 min is needed to stain conidia intense blue) to ensure that the appropriate density of conidia is achieved. Conidia concentration and the amount of debris interfering with counting and identification can vary greatly. In some cases, it will be necessary to prepare another filter from the same sample and adjust the volume filtered or make a dilution.

8. At least 10 microscopic fields (*ca.* 1 mm^2 each) and at least 200 conidia should be counted and identified at 150–200× magnification. Use a modification of Table 15.1 (not provided) to enter results. A substantially lower number of conidia is acceptable for early (less than a week) and late stages of leaf litter decomposition since sporulation rates are typically very low at these times.

Data Analysis

1. Sporulation rate S (conidia mg^{-1} AFDM d^{-1}) is calculated as:

$$S = \frac{n \cdot A_e \cdot V_c}{f \cdot A_{mf} \cdot V_a \cdot m \cdot t} \qquad (15.2)$$

where n is the number of conidia counted; A_e, effective filtered area (mm^2); V_c, volume of fluid in chamber (mL); f, number of fields counted; A_{mf}, microscopic field area (mm^2); V_a, aliquot of conidia suspension filtered (mL); m, AFDM of 10 disks used to induce sporulation (mg); t, incubation time (d).

2. Conidia production can also be expressed in terms of dry mass or fungal carbon (Baldy *et al.* 2002, Gulis and Suberkropp 2003b) using published values on dry masses of conidia of dominant aquatic hyphomycetes (Chauvet and Suberkropp 1998) or biovolume estimates (Bärlocher and Schweizer 1983).

C. Advanced Method 1: Fungal Biomass

Preparation Protocol

1. Prepare ergosterol standards in methanol (e.g., in the range of 2-25 µg/mL) and store at −20°C.
2. Prepare alcoholic KOH solution. Dissolve 4.8 g KOH in 6 mL distilled water. When KOH has dissolved, add 114 mL methanol, which is sufficient for 20 samples.

Field Protocol

1. Collect leaf litter as described in Basic Method 2.
2. For each sample cut 2 sets of 5 leaf disks each. Preserve one set in 5 mL of methanol in a 20-mL glass scintillation vial. Transport to the laboratory in a cooler and then store in a freezer (−20°C) for up to several months. Use the corresponding set of leaf disks for AFDM determination.

Laboratory Protocol

1. Determine AFDM of the corresponding set of disks as described in Basic Method 2.
2. *Ergosterol extraction*. Transfer leaf disks and 5 mL of methanol in which they were stored to round bottom flasks. Use 5-mL aliquots of methanol to wash vials. Make final volume to 25 mL. Add 5 mL of alcoholic KOH and a boiling chip.
3. Attach flasks to reflux condensers, turn on water to condensers, and place flasks in the water bath at 70°C. Reflux for 30 min.
4. Remove flasks from the water bath and let cool. Remove and dispose of leaf disks and boiling chips using forceps. Transfer extract from each flask to a 65-mL screw-top tube and add 10 mL of deionized water.
5. Use 10 mL of pentane to wash each round bottom flask and add to methanolic extract in screw-top tube. Screw cap on tightly. Mix thoroughly by inverting tubes at least 30 times (or in a Rotamix for 3 min at 20 rpm). Remove pentane (upper phase) with a Pasteur pipette into a 15-mL conical centrifuge tube. In a fume hood, evaporate pentane in tube heater at 30°C under a stream of N_2 delivered through syringe needles.
6. Repeat the partitioning into pentane step twice by adding 5 mL of pentane to methanol phase in the screw-cap tube, mixing, removing top phase to the same centrifuge tube, and evaporating as above. Discard methanol phase.
7. Evaporate pentane to dryness and redissolve residue in 1 or 2 mL of methanol (use a precise syringe, as accuracy is critical). Sonicate in bath sonicator (twice for 5 min or until no visible residue on tube walls).
8. Filter ergosterol extract through 0.2-µm syringe filter (PTFE) into HPLC vial. Extracts can be stored at −20°C for several months until analyzed by HPLC.
9. *HPLC purification and quantification of ergosterol*. Sonicate extracts to degas (5 min) after removal from a freezer. Set a UV detector to 282 nm. Use methanol as a mobile phase and set flow rate to 1.0–1.5 mL/min. Inject 20 µL of each ergosterol standard followed by sample extracts. Retention time of ergosterol varies depending on flow rate, temperature, and column properties and usually is 4–10 min. Use peak area of ergosterol standards to perform regression and calculate ergosterol concentrations of samples.

Data Analysis

1. Fungal biomass B_f (mg/g AFDM) associated with plant litter can be calculated as:

$$B_f = \frac{C_e \cdot V_e}{m \cdot 5.5} \tag{15.3}$$

    where C_e is ergosterol concentration of sample extract from HPLC (µg/mL); V_e, volume of extract (i.e., 1 or 2 mL); m, AFDM of corresponding set of 5 leaf disks (mg); 5.5 is ergosterol to biomass conversion factor (5.5 mg ergosterol/g fungal dry mass) (Gessner and Chauvet 1993).
2. If community structure of aquatic hyphomycetes is known (Basic Method 2), species-specific conversion factors may be applied to calculate fungal biomass (Gessner and Chauvet 1993, Baldy *et al.* 2002). It is not clear, however, whether conidial production of individual species from leaf litter correlates with their contribution to mycelial biomass within substrates.

D. Advanced Method 2: Fungal Production

Preparation Protocol

1. Prepare [1-^{14}C]acetate plus nonradioactive sodium acetate stock solution. The stock solution is made so that 50 µL contains 1 MBq of [^{14}C] acetate and the total acetate concentration is $0.4\,M$.
2. Filter stream water through a membrane filter (0.45 µm pore size) and distribute 3.95 mL to each incubation tube equipped with an aeration tube.

Field protocol

1. Collect leaf litter as described in Basic Method 2.
2. For each sample, cut two sets of 5 leaf disks each. Place one set in an incubation tube containing 3.95 mL of filtered stream water and use the corresponding set for AFDM determination.
3. One extra tube containing leaf disks will be used as the killed control to determine background levels of radioactivity in the ergosterol fraction. After leaf disks have been placed in this tube, add formalin to a final concentration of 2%.
4. Place tubes in a rack in the stream, connect aeration tubes to a battery operated pump and aerate each tube with 30–40 mL air/min. If handling radioactivity in the field is not possible, then tubes and disks should be placed in a cooler close to stream temperature and transported back to the laboratory where the tubes can be placed in a water bath adjusted to stream temperature in a fume hood and aerated.
5. Allow the disks to equilibrate for 10–20 min and add 50 µL of [^{14}C]acetate solution to each tube at timed intervals and incubate for a precise time (120–300 min).
6. At timed intervals, remove the tubes from the stream in the same order as addition of acetate occurred and place in an ice bath to slow additional uptake of acetate.

7. Filter the contents of each tube through glass fiber filters (25 mm diam). Rinse with filtered stream water and place filters and leaf disks in 5 mL methanol. Transport to the laboratory on ice and store at −20°C until ergosterol is extracted.

Laboratory Protocol

1. Extract and measure ergosterol as described in Advanced Method 1 with modifications described below. Make certain to follow proper protocol for handling radioactive samples and waste.
2. Keep the final volume of the ergosterol extract as small as possible (0.5–1.0 mL) and inject larger volumes (100–250 μL) into the HPLC than used for ergosterol determination (Advanced Method 1). Multiple injections of the same sample are also recommended. All these precautions increase the sensitivity of the assay since the radioactivity of the ergosterol fraction is typically very low.
3. Collect the ergosterol peak eluting from the HPLC in a scintillation vial.
4. Add 10 mL of scintillation fluid to the combined ergosterol fractions from each sample.
5. Determine radioactivity of ergosterol with a scintillation counter and correct for quenching.

Data Analysis

1. Calculate ergosterol concentrations from peak areas and standard curve as in Advanced Method 1.
2. Calculate the fungal growth rate (k) (mg mg^{-1} d^{-1}) as:

$$k = \frac{(R_s - R_c) \cdot 19300}{a_s \cdot F \cdot t \cdot B_{fs}} \tag{15.4}$$

where R_s is the radioactivity (Bq) in the sample; R_c, radioactivity (Bq) in the control; a_s, specific activity (Bq/mmol) of the acetate; F, fraction of the sample that is injected into the HPLC; t, incubation time (d); B_{fs}, fungal biomass in the sample (mg); 19300 is an empirically derived conversion factor (mg fungal biomass/mmol acetate incorporated) (Suberkropp and Weyers 1996). See Gessner and Newell (2002) for other conversion factors.

3. Fungal production (P_f) (mg g^{-1} leaf AFDM d^{-1}) is calculated as:

$$P_f = k \cdot B_f \tag{15.5}$$

where k is the growth rate (mg mg^{-1} d^{-1}) and B_f is the fungal biomass (mg/g leaf AFDM).

IV. QUESTIONS

1. What stream characteristics affect concentrations of aquatic hyphomycete conidia in water?
2. Why does conidia concentration in stream water usually exhibit a clear seasonal pattern? Would you expect to find seasonal pattern in tropical, arctic, or desert streams? Would you expect to find diel fluctuations in conidia concentration?
3. Are species identified from water samples similar to those recorded from laboratory sporulation experiments from the same stream? Why or why not?
4. Why is it important to use filtered stream water for laboratory incubations to induce sporulation of aquatic hyphomycetes?
5. Did you notice significant differences in sporulation rates of aquatic hyphomycetes from different types of leaf litter (substrates)? Why or why not?
6. How does nutrient concentration in water, siltation, or algal development affect fungal activity on submerged plant litter?
7. What interactions may exist between aquatic fungi and stream invertebrates?
8. What implications do riparian management practices or land use in the watershed (logging, agriculture, urban development, etc.) have on fungal activity in streams?

V. MATERIALS AND SUPPLIES

Letters in parenthesis indicate in which Basic (B1 or B2) or Advanced (A1 or A2) Method the item is used.

Field Materials

[1-^{14}C]acetate, sodium salt, and nonradioactive sodium acetate (A2)
Acid-washed plastic bottle (B2)
Air flow meters (A2)
Battery-operated air pump (A2)
Cork borer (e.g., 11.2 mm diam.) (B2, A1, A2)
Filter holders to accommodate 47-mm diam. filters (B1) and 25-mm diam. filters (A2)
Formalin (A2)
Glass fiber filters (25-mm diam.; e.g., Whatman 934-AH) (A2)
Glass scintillation vials (20 mL) (A1, A2)
Graduated cylinder (500 mL) (B1)
Hand pump with manometer (B1)
Ice (A2)
Membrane filters (47-mm diam., 5–8 μm pore size; e.g., Millipore, white SMWP) (B1)
Petri dishes with tight-fitting lids (50-mm diam.; e.g., BD Falcon) (B1)
Receiving flasks (B1, A2)
Stain dropper (B1)
Sterile filter apparatus and membrane filters (0.45 μm pore size) (A2)
Forceps (B1, B2, A1, A2)
Trypan blue (Sigma) in lactic acid (0.05%) or cotton blue (Sigma) in lactic acid (0.1%) (B1, B2)

Tubes (40 mL) fitted with 2-holed rubber stoppers, glass and rubber tubing for aeration, and a tube rack (A2)

Wide-mouth jars (50 mL) (B2)

Ziplock bags (optional) (B2, A1)

Laboratory Materials

Beakers (100 mL) (B2)

Conical glass centrifuge tubes with screw caps (15 mL) (A1, A2)

Ergosterol (purity ≥98%; e.g., Alfa Aesar)

Filter holder to accomodate 25-mm diam. filters (B2)

Glass fiber filters (Whatman GF/F) (B2)

Glass scintillation vials (20 mL) (A2)

Glass syringes (0.5, 1, 5 mL) (A1, A2)

HPLC vials with teflon-lined caps (2 mL) (A1, A2)

KOH (A1, A2)

Membrane filters (25-mm diam, 5–8 μm pore size; e.g., Millipore, white SMWP) (B2)

Methanol (HPLC grade) (A1, A2)

Microscope slides and coverslips (22×22 mm and 22×40 mm) (B1, B2)

Receiving flask (1 L) (B2)

Round bottom flasks (100 mL) (A1, A2)

Reflux condensers (A1, A2)

Pentane (HPLC grade) (A1, A2)

Scintillation fluid (e.g., Ecolume) (A2)

Screw top tubes with teflon lined caps (65 mL) (A1, A2)

Small crucibles or aluminum weighing boats (B2, A1, A2)

Syringe filters (13-mm diam, 0.2 μm pore size, PTFE) (A1, A2)

Teflon boiling chips (A1, A2)

Forceps (B1, B2, A1, A2)

Triton X-100 (Sigma) solution (0.5%) (B2)

Trypan blue in lactic acid (0.05%) or cotton blue in lactic acid (0.1%) (B1, B2)

Laboratory Equipment

Analytical balance (B2, A1, A2)

Adjustable pipettors (200 μL, 1 mL, and 5 mL) (B2, A1, A2)

Compound light microscope (B1, B2)

Drying oven (B2, A1, A2)

Dry tube heater (A1, A2)

HPLC system [pump for isocratic operation, autosampler, or 20 μL injection loop (A1) or 100 μL injection loop (A2), UV detector, integrator, or computer with software package (A1, A2) and automatic fraction collector (A2)]

HPLC column (reverse phase C_{18}; e.g., 25×4.6 mm, Whatman Partisphere) (A1, A2)

Magnetic stirrer and stirring bars (B2)

Muffle furnace (B2, A1, A2)

Rotating tube mixer (Rotamix) (A1, A2)

Scintillation counter (A2)

Sonication bath (A1, A2)

Tank of nitrogen gas and manifold with syringe needles (A1, A2)

Vacuum pump/connection (B2)

Water bath (70°C) (A1, A2)

VI. REFERENCES

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Benthic Stream Algae: Distribution and Structure

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I. INTRODUCTION

The algae are a ubiquitous group of photosynthetic organisms responsible for the majority of photosynthesis in most sunlit streams. Benthic algae are dominant members of the periphyton community living on submerged substrata in the photic zone of most aquatic ecosystems including both marine and fresh water. Although algae are usually studied as a group of organisms that are closely related at a functional level, they are very diverse on an evolutionary level and have been assigned to several different kingdoms based on morphological, chemical, and ecological parameters.

An alga (singular) or algae (plural) are organisms lacking true tissues and multicellular gametangia and containing chlorophyll a, and their colorless relatives. This definition excludes higher plants (macrophytes) such as *Potamogeton*, *Lemna*, and other flowering aquatic plants that have well developed true tissue and organ systems. The definition also excludes aquatic bryophytes (mosses and liverworts), which have multicellular gametangia, and aquatic fungi which have no chlorophyll. Since the group of organisms classified as algae is ecologically closely related but evolutionarily distantly related, algae are a taxonomically "unnatural" group of organisms. Thus, some algae, such as the blue-green algae, are also bacteria (cyanobacteria), while others are more similar to animals (dinoflagellates and chrysophytes). Dinoflagellates are also protozoa, and most algae are also considered to be protista. Only one group of algae is true plants in the evolutionary sense (green algae), but because all algae possess chlorophyll *a* and liberate oxygen

during photosynthesis, botanists have traditionally studied them. The lack of taxonomic cohesiveness is often frustrating to aquatic researchers who prefer clean, unambiguous systems of classification. Still, algae share many physiological, morphological, and ecological features that make them a logical group of aquatic organisms to investigate as a community. In this chapter we illustrate how to (1) collect and identify benthic algae from lotic habitats and (2) investigate microhabitat fidelity among the algae.

A. Classification

Algae can be classified in several different ways. We will focus on two different classification schemes: taxonomic classification and ecological classification.

Taxonomic Classification. Algae belong to at least ten different taxonomic divisions (divisions in plant taxonomy are equivalent to phyla in zoological classification). Classification is based on four major considerations: (1) pigmentation (kinds and quantities), (2) internal storage products (chemistry and structure), (3) cell wall (chemistry and structure), and (4) flagellation (number and type). All of the above taxonomic parameters are considered to be evolutionarily conservative and are thus good tools for recognizing divisions of taxonomically related algae. Taxonomic differences among the five major divisions of algae common in periphyton communities of freshwater streams are presented in Table 16.1.

TABLE 16.1 **Patterns of Pigment Content, Cell Wall Chemistry, Storage Chemistry, and Flagellation Among the Divisions of Algae Most Commonly Encountered in Freshwater Periphyton.**

Division	Pigmentation	Cell Wall	Storage Products	Flagellae
Bacillariophyta (diatoms)	Chlorophylls *a* and *c* but with carotenoid pigments dominant; cells usually gold to brown in color	Mostly SiO_2 and composed of two overlapping halves	Oil and leucosin	Absent vegetatively
Chlorophyta (green algae)	Chlorophylls *a* and *b* dominant	Cellulose and pectin	Plant starch	Usually 2–4 of equal length when present
Cyanophyta (blue-green algae)	Chlorophyll *a* and phycobilins; blue-green to olive-green in color	Peptidoglycan, gram-negative	Glycogen-like	Absent
Chrysophyta (yellow-green algae)	Chlorophylls *a* and *c*; yellow-green in color	Pectin and cellulose	Oil and leucosin	Absent vegetatively
Rhodophyta (red algae)	Chlorophyll *a* and phycoerythrin; olive-green to maroon in color	Mannans and xylans (slimy)	Glycogen-like	Absent

Diatoms (Bacillariophyta) are probably the most widespread and abundant of all divisions of benthic algae. They are recognizable in the field where they form buff to brown to gold colored films on submerged objects. Under microscopic examination, the two cell walls of a diatom (called valves) fit together like the halves of a Petri dish to form the frustule, or siliceous cell wall. The valves are penetrated by many pits and pores (punctae) symmetrically arranged in rows (striae). Many diatoms possess a slit through the cell wall (raphe) that allows them to be motile (Appendix 16.3-Fig.1). Diatoms are classified to genus primarily on the basis of symmetry and cell ornamentation.

Green algae (Chlorophyta) in the periphyton are usually filamentous with sub-spherical to cylindrical cells attached end to end. In the field, green algae have a color similar to green terrestrial plants. Filaments of green algae may be branched or unbranched. Occasionally, non-filamentous cells or colonies of green algae also may be present in periphyton communities but are rarely dominant (Appendix 16.3-Fig.2).

Blue-green algae (Cyanophyta) are also correctly called Cyanobacteria because of their prokaryotic nature. They may appear as mats of olive-green to blue-green to brown growth and often have a characteristic musty odor. Filamentous forms are most common in benthic habitats.

Yellow-green algae (Chrysophyta) are represented by only a few genera in the periphyton (*Vaucheria*, *Hydrurus*, and *Tribonema*). All three of these genera are filamentous and may become locally abundant.

Red algae (Rhodophyta) are most abundant in oceans but several filamentous genera occur in fresh water. They usually appear olive-green to maroon in color.

Ecological Classification. Algae can colonize almost any submerged substratum and can be classified according to the microhabitat that they occupy. Epilithon is the name given to benthic algae growing on rocks, epiphyton grows on plants (including filamentous algae), epidendron grows on wood, epipelon grows on fine sediment, epipsammon grows on sand, and epizoon grows on aquatic animals. Finally, algae that are only loosely associated with the substratum, such as cloudy masses of *Spirogyra*, are called metaphyton. It should be noted that substratum availability and abundance is a function of other stream variables such as current velocity, catchment geology, and the nature of terrestrial riparian vegetation. Within each microhabitat, if it is present in the stream of interest, there is a considerable amount of algal microhabitat specificity as algal specialists have evolved to occupy specific microhabitats. These microhabitat specialists are often the same across similar streams within a region. For example, epipelic microhabitats are usually dominated by highly motile diatoms capable of moving over and between fine particles of sediment. On the other hand, firmly attached diatoms or green filamentous algae usually dominate epilithon.

B. Physiognomy

Algal community physiognomy addresses the physical or architectural structure of the community. Benthic algae can develop a complex physical structure similar to that of a terrestrial forest (Hoagland *et al.* 1982) except on a much smaller scale (Appendix 16.3-Fig.3). Different algal growth forms include: non-motile and prostrate; attached by a mucilage pad at one end; attached to the end of long mucilaginous stalks; or filamentous. Some species are motile and travel throughout the structured community. Benthic algal communities on different substrata develop different physiognomies, which can affect interspecific

interactions among the algae. Physiognomy also can impact the nature of interactions between benthic algae and stream invertebrates (Steinman *et al.* 1992).

C. Roles of Benthic Algae in Stream Communities

Benthic algae play several roles of fundamental importance to stream ecosystems. As organisms at the base of the food web, they are at the interface of the physical-chemical environment and the biological community. Photosynthesis by benthic algae provides oxygen for aerobic organisms in the ecosystem and the fixed carbon provides food for algivores. In many stream habitats, the contribution of organic carbon to the food web from algal photosynthesis is considerable (Lamberti 1996, Steinman 1996). Benthic algae may enter the food web through direct consumption from the substratum by benthic invertebrates such as snails, insects, or protozoa (Steinman *et al.* 1987, Barnese *et al.* 1990, Bott and Borchardt 1999), or drifting benthic algae may be captured by filter feeders that strain the water column (Barnese and Lowe 1992).

A second role of benthic algae that stems from their position at the interface of abiotic and biotic stream components is their utility as water quality indicators (Lowe and Pan 1996, Stevenson and Smol 2003). Benthic algae possess many attributes that make them ideal organisms to employ in water quality monitoring investigations. Because benthic algae are sessile, they cannot swim away from potential pollutants. They must either tolerate their surrounding abiotic environment or die. Benthic algal communities are usually species-rich, and each species, of course, has its own set of environmental tolerances and preferences (Lowe 1974, Beaver 1981, Van Dam *et al.* 1994). Thus, the entire assemblage represents an information-rich system for environmental monitoring. The short life cycles of most benthic algal species result in a rapid response to shifts in environmental conditions. Extant benthic algal communities are typically quite representative of current environmental conditions. Identification is not exceedingly difficult. Taxonomy of benthic algae is usually based on cell or thallus morphology and easily discernible through the light microscope. Excellent taxonomic keys exist for identification of benthic algae in most parts of the world.

The objective of this chapter is to introduce stream benthic algae to the investigator who has little previous experience with algae. You will learn how to recognize periphyton growth in the field in a variety of microhabitats. You will learn how to identify dominant algal taxa under the microscope. Finally, you will learn how to estimate algal density on a substratum and calculate community parameters based on two measures of algal abundance: cells/unit area and biovolume/unit area.

II. GENERAL DESIGN

A. Site Selection

As just mentioned, benthic algae grow on submerged substrata that receive ample sunlight. In large relatively clear rivers this may include substrata several meters deep. However, we will focus on wadeable stream sections in this exercise. Try to select a stream reach that has a variety of substratum types to maximize the types of benthic algae that you will see. Ideally, the stream reach would include examples of epilithic, epidendric, epipelic, epiphytic, epipsammic, and (if you're fortunate) epizoic habitat. Stream reaches including all of these substratum types are unusual, but streams usually contain from one to four microhabitats.

B. Field Sampling

Epilithon. Epilithic habitats are usually found in areas of the stream that experience relatively fast current. Thus, epilithic algae are often tightly attached to the substratum. This fact necessitates scraping rock substrates with a knife, scalpel, or similar tool (see Figure 17.1 for an example). In general, the stone should be removed from the stream before scraping so that scraped algae aren't washed away. If the stone is too large to remove from the stream, the benthic algae can be scraped from the stone under water and captured in a small plankton net as algae are washed downstream.

Epidendron or Epixylon. Epidendric or epixylic habitats may take the form of woody debris from riparian vegetation or from submerged woody tissue of living vegetation such as alder or willow trees. The best means for collecting this community is similar to methods for collecting epilithon.

Epipelon. Epipelic algae are often motile and only loosely associated with the substratum. Since epipelic habitats occur in areas of little or no current where fine sediment can accumulate, one need not worry about the benthic algae washing away while collecting. Epipelon is best collected with a turkey baster or with a pipette and rubber bulb. Care must be taken not to penetrate the sediments too deeply with the pipette or baster. Most of the benthic algae will be on top of the sediment or within the first millimeter. Collection of deeper layers will obscure live cells during microscopic analysis. This community can be collected quantitatively by defining the area on the sediment to be collected. A rubber hose washer or O-ring works nicely to isolate the sediment surface area for quantitative collections.

Epiphyton. Epiphytic algae are usually tightly attached to their plant hosts (filamentous algae or aquatic macrophytes). A small portion of the plant host should be placed into a bottle with a small amount of stream water leaving air space in the top of the bottle. The bottle should then be shaken vigorously to remove the epiphytes from the plant host. The host can then be removed from the bottle, wrung out and discarded. This procedure will leave some tightly attached epiphytes remaining on the plant host. These can be observed directly by mounting a portion of the plant epidermis on a microscope slide and studying the epiphytes while they are still attached to the host.

Epipsammon. As with epiphyton, a small quantity of the sand substrate should be agitated in a bottle containing water. The sand will quickly fall to the bottom of the bottle after agitation and the suspended algae can be poured off the top into another container. As with plant epidermis, sand grains are usually transparent enough to be observed directly under the microscope. This technique allows the investigator to study the microdistribution of algae on individual sand grains.

Epizoon. Epizoic algae are most likely to be collected from larger animals with a rigid covering such as snails, clams and turtles. Algae should be scraped from these organisms as if they were epilithic habitats. Smaller animals such as midges (Pringle 1985) and caddis flies (Bergey and Resh 1994) can also host unique epizoic algal floras. These habitats can be investigated by carefully collecting the host and observing sections of its retreat directly under the microscope.

C. Preservation and Labeling

Samples should be transferred into plastic vials or bottles which are labeled externally with a waterproof pen. It is a good idea to place a small label into the collection vial as well in case samples are later transferred to a different container. The label should include information about the date, stream and microhabitat and surface area sampled for quantitative samples. Both formaldehyde and glutaraldehyde at a final concentration of 3 to 5% will preserve samples well for later examination. In addition to labeling samples directly, a field note book should also be annotated with details about each collection identified by collection number. A workable system for numbering samples is the month-day-year-collection number method. For example, sample 12-28-05-7 would be the seventh sample collected on December 28, 2005. Information entered into the notebook should include field observations such as water temperature, pH, depth, substratum type, color of growth collected, and any other information that seems pertinent to the collection.

D. Laboratory Processing

The algal samples can be examined directly in a wet mount with a compound microscope but in order to identify diatoms to genus they must first be "cleaned" (Van Der Werff 1955). This process involves oxidizing the organic matter in the sample so that just the silica cell walls of diatoms remain. The empty valves and frustules are then mounted in a mounting medium of high refractive index such as Naphrax®. A detailed step-by-step procedure for cleaning and mounting diatoms is presented in Appendix 16.1.

Permanent slides of "soft" algae, as nondiatom algae are often called, also can be made quite easily and inexpensively but it is not necessary for soft algal identification. An easy and inexpensive technique for preparing semipermanent slides of soft algae is the modification by Stevenson (1984) of Taft's (1978) glucose mounts as presented in Appendix 16.2.

Identification of Algae. Collections of algae from the field should be mounted in a wet mount on a microscope slide, covered with a cover glass and examined on a compound microscope. It is best to first examine the slide with relatively low magnification (about 100X) to get comfortable with the range of taxa and types of morphologies present. After a few minutes, switch to higher magnification (430–450X) and continue to examine the collection. When you are comfortable with the microscope and have some good representative specimens, begin to key out some of the most common taxa. A simplified key to genera of stream algae is presented in Appendix 16.3. This key does not include all of the genera found worldwide but instead focuses on dominant genera of rivers in North America. As you work through the key, the first few times take notes on which dichotomy you chose. You may have to backtrack if you go the wrong way in the key. If you have chosen a rare or uncommon alga to key out, it may not be in this key and you will reach a dead end. Several more comprehensive keys are available for more in-depth investigations into riverine algae (see Appendix 16.4).

Quantitative Methods. Benthic algae can be enumerated in a quantitative fashion that allows the estimation of cells of taxa per cm^2 of substratum. Field collection techniques

are the same as detailed above except that the surface area sampled is measured and recorded. This is most easily done on flat surfaces of stones where a section (2 to 10 cm^2) can be isolated from the rest of the stone with duct tape.

In the laboratory, the sample volume is measured and a subsample of the suspension is pipetted into a Palmer-Maloney® nanoplankton counting chamber. This is a device that is 0.4 mm deep and holds exactly 0.1 mL of suspension. The volume of sample contained in a single microscope field in the counting chamber (V_f) can be calculated by the formula:

$$V_f = \pi r^2 d \tag{16.1}$$

where r is the radius of the microscope field and d is the depth of the counting chamber (0.4 mm).

The number of fields examined in the counting chamber to quantify benthic algae is a function of the density of algae in the sample but it is customary to examine enough fields to enumerate 300–500 algal cells. The cell density of ith algal taxon (D_i) is calculated by the following formula:

$$D_i = N_i V_s / V_c / A \tag{16.2}$$

where D_i is the density of cells of the, N is the number of cells of the ith taxon counted in the counting chamber, V_s is the total volume of the sample, V_c is the volume of the sample counted [(fields counted)(V_f)], and A is the area in cm^2 of substratum sampled.

The total density of benthic algae on the substratum can be calculated by summing D_i for all 1 through n taxa encountered. One can also report algal densities on the basis of biovolume/cm^2. This may be a more appropriate measure of the success of an algal population than cell number since a single cell of one species may be over 1000 times larger than the cell of a second species. Biovolumes can be calculated simply by measuring cells with a calibrated ocular micrometer and using appropriate geometric formulae to calculate their volumes. Most cells can be viewed as cylinders, cones, spheres, or elongated cubes for purposes of biovolume estimates (Wetzel and Likens 1991). Several cells (5–10) from each population should be measured to obtain an average biovolume for each algal taxon of interest. The density of an alga based on biovolume is the product of its biovolume per cell and its cellular density.

Palmer-Maloney counting chambers are relatively expensive and it is possible to make your own chamber by pounding a clean hole through a plastic cover slip with a cork borer and cementing the cover slip to a microscope slide. The depth of the well inside the cored cover slip can be calculated with precision calipers.

III. SPECIFIC METHODS

A. Basic Method 1: Investigation of Algal Microhabitats

As discussed above, algae occupy a variety of microhabitats within stream systems. In this exercise microhabitats will be sampled qualitatively to examine the role of habitat variables in the distribution of algal taxa.

Field Sampling

1. Select a stream reach that provides a variety of substratum types and a range of physical variables such as light quantity and current speed. A variety of current speeds can be obtained, for example, by sampling different sides of a boulder. Different light intensities can be obtained by sampling inside or outside shady patches resulting from stream-side vegetation.
2. Select five to eight different microhabitats from which to collect benthic algae.
3. Collect and preserve benthic algal samples in the field while taking detailed notes concerning each microhabitat. Remember, this is a microbial community and microhabitats are often quite small. Each collection should be limited to a few cm^2.

Laboratory Identification

1. After returning to the laboratory, examine the collections from each microhabitat to make certain that you have collected a healthy benthic algal community. If specimens are mostly dead or if the sample contains too much debris to view algae clearly, discard the sample. Bad collections can be avoided by closely examining the microhabitats and collecting carefully.
2. Make permanent diatom mounts and semi-permanent glucose mounts from each collection.
3. Identify the five most numerically abundant algal genera in each sample and record them on the Benthic Algal Survey Worksheet (Table 16.2).

B. Advanced Method 1: Analyses of Algal Density: Cellular versus Volumetric Analyses

It is often useful to determine the absolute abundance of algal taxa on a specific substratum. In this exercise, epilithic benthic algal communities from two contrasting current speeds will be compared quantitatively.

Field Sampling

1. In the field, select pool and riffle habitats that are representative of the stream.
2. Carefully remove three stones from the pool and three stones from the riffle for quantitative benthic algal analysis. Try to select flat-topped stones whose surfaces lie parallel to the stream bed.
3. On the stream bank, isolate $4\,cm^2$ of each of the stone surfaces by overlapping four strips of duct tape at right angles (or use a pre-made template) to leave a 4-cm^2 opening in the center.

TABLE 16.2 **Worksheet for Benthic Algal Survey: Investigations of Algal Microhabitats.**

Stream:	Date:	Location:		Investigators:
Microhabitat Type		Notes About Microhabitat (Current velocity? Color of growth? Sunny or shaded?)		Dominant Algal Genera
1.				1a.
				1b.
				1c.
				1d.
				1e.
2.				2a.
				2b.
				2c.
				2d.
				2e.
3.				3a.
				3b.
				3c.
				3d.
				3e.
4.				4a.
				4b.
				4c.
				4d.
				4e.
5.				5a.
				5b.
				5c.
				5d.
				5e.

4. Brush and scrape benthic algae from each isolated surface into a collecting basin using a double-edge razor blade and a firm-bristle toothbrush.
5. Flush the sample into a bottle and preserve it. This procedure will result in three replicate samples from each pool and riffle habitat.

Laboratory Identification

1. In the laboratory bring all samples to an equal volume (between 20 and 50 mL).
2. Transfer a subsample of the sample into a counting chamber and examine enough fields to identify 300 algae to genera. A bench sheet (Table 16.3) is provided for data entry.

TABLE 16.3 Worksheet for Analyses of Algal Density: Cellular versus Volumetric Analyses.

Stream:	Date:	Location:	Investigators:
Microhabitat Type	Dominant Algal Taxa	Cells·cm^{-2}	Biovolume·cm^{-2}
Riffle, stone 1	R1a.		
	R1b.		
	R1c.		
	R1d.		
	R1e.		
Riffle, stone 2	R2a.		
	R2b.		
	R2c.		
	R2d.		
	R2e.		
Riffle, stone 3	R3a.		
	R3b.		
	R3c.		
	R3d.		
	R3e.		
Pool, stone 1	P1a.		
	P1b.		
	P1c.		
	P1d.		
	P1e.		
Pool, stone 2	P2a.		
	P2b.		
	P2c.		
	P2d.		
	P2e.		
Pool, stone 3	P3a.		
	P3b.		
	P3c.		
	P3d.		
	P3e.		

3. Each researcher may work on a different sample for data generation with the results of individual enumerations averaged for each habitat.
4. Calculate cells/cm^2 of the five most abundant algal taxa.
5. Determine the average biovolume/cell of the abundant taxa and calculate biovolume/cm^2 of each of the same taxa.
6. Compare differences in dominant taxa between habitats based on cell number. Compare differences in dominant taxa between habitats based on cell biovolume.

IV. QUESTIONS

Basic Method 1

1. How does current speed affect the availability of substratum types for benthic algal colonization?
2. Which microhabitat has the most filamentous green algae? Why?
3. Where are motile (raphe-bearing) diatoms most abundant? Why would motility have an adaptive advantage in this microhabitat? Where would motility be a disadvantage?
4. Do different divisions of algae seem to do better in well-illuminated or shaded microhabitats? Why?
5. How do you think grazers affect the patterns of algal distribution that you found?

Advanced Method 1

1. Do different algal genera dominate pools and riffles?
2. Are algal densities of pools different than densities of riffles? Why?
3. What is the relationship between the size and the numerical abundance of an algal taxon?
4. What do you feel is a better measure of the success of an algal taxon, cells/cm^2 or biovolume/cm^2? Why?
5. In what circumstances might one measure have more ecological relevance than the other? Why?

V. MATERIALS AND SUPPLIES

Field

> Knife, scalpel, or double-edged razor blades
> Toothbrush
> Plankton net
> Turkey baster or medicine dropper
> 10 collection bottles (10–50 mL, plastic preferable)
> Waterproof field notebook and pencil

Laboratory

> Microscope
> Slides and cover glasses
> 30% hydrogen peroxide

Granular potassium dichromate
Glutaraldehyde
Formaldehyde
Naphrax® mounting medium
Corn syrup
Hot plate
Nail polish
Glass beakers (1000 mL and 250 mL tall)

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APPENDIX 16.1
Cleaning and Mounting of Diatoms (modified from Van Der Werff 1955)

This procedure should be performed in a fume hood or out of doors. Hydrogen peroxide is a strong oxidant! The investigator should wear eye protection, a laboratory coat, and protective gloves.

1. Place a small amount of the sample to be cleaned in a 1000-mL beaker. Five or 10 mL of sample is usually adequate.
2. Add about 80 mL of 30% H_2O_2 and allow the mixture to stand for 24 hours. If necessary, you can proceed to step 3 without waiting 24 hours, but oxidation of organic matter may be incomplete.
3. Add a micro-spatula of $K_2Cr_2O_7$ to the mixture in a fume hood. This will initiate a violent exothermic reaction. At the completion of the reaction, 5 to 10 minutes, the solution will change from purple to a golden color.
4. Transfer the solution to a tall 200 mL beaker and add distilled water until the beaker is full.
5. Allow the mixture to stand for a minimum of 4 hours as the cleaned diatoms settle to the bottom. Decant the mixture, carefully removing and discarding the liquid but being careful not to disturb the diatom-cell sediment on the bottom of the beaker. About 30 mL of the mixture should remain in the beaker. Refill the beaker with distilled water.
6. Repeat step 5 until the mixture is colorless.
7. Pipette a portion of the 30-mL concentrate onto an alcohol-cleaned cover glass and air dry.
8. Place a drop of mounting medium on a clean microscope slide and invert the cover glass on the drop.

9. Heat the slide on the "high" setting on a laboratory hot plate for about 30 seconds or until the bubbling of the medium slows.
10. Remove the slide and by applying gentle pressure with forceps force air bubbles from beneath the cover glass.
11. Allow the slide to cool and label with collection number.

APPENDIX 16.2
Making Semipermanent Mounts of Soft Algae

Stock Solutions

A. 4% Formaldehyde
B. 100% Taft's Glucose: (7 parts 4% Formaldehyde solution + 3 parts light Karo® corn syrup).
C. 10% Taft's Glucose: (9 parts H_2O + 1 part 100% Taft's Glucose solution)

Procedure

1. Preserve material in approximately 2% glutaraldehyde fixative. (Note: Glutaraldehyde is a strong fixative with little noticeable odor; use it carefully and keep it away from your own cells.)
2. Place material with fixative onto a cover slip. Add 10% Taft's Glucose solution (Material + fixative: 10% Taft's approximately 1:1).
3. Allow material to dry to tackiness.
4. Place a drop of l00% Taft's Glucose onto a microscope slide. Invert the dried material on cover slip onto slide.
5. Lightly press cover slip to evenly spread the mountant under cover slip.
6. Allow the material to harden. If the glucose solution pulls away from the edge of cover slip add additional 100% Taft's Glucose solution.
7. Ring and seal with fingernail polish along all four edges of cover slip.

APPENDIX 16.3
Most Common Lotic Algal Genera

1a. Pigments in cells localized in chloroplasts and not diffused throughout the cell; pigments usually some shade of green or gold ------------------------------4

1b. Pigments diffuse in the cells and not localized in chloroplasts; cells often very small and olive-green to blue-green to brownish in color ----------------- Blue-Green Algae --- 2

2a. Filamentous --- 3

2b. Not filamentous ----------------------- *Chroococcus*, *Microcystis*, *Aphanothece*, *Merismopedia*, or closely related genus (Figure 4)

3a. Filaments composed of cells that all look alike --------- *Oscillatoria*, *Phormidium*, *Schizothrix*, *Lyngbya,* or related genera (Figure 5)

3b. Filaments with some cells that look different (heterocysts)------*Anabaena*, *Nostoc*, *Tolypothrix*, *Calothrix*, or related genera (Figure 6)

4a. Chloroplasts grass-green, alga may be filamentous, single-celled or colonial------5

4b. Chloroplasts not grass-green but yellow-green, olive-green, gold, brown or pink; may be filamentous, single-celled or colonial ------------------------------23

5a. Alga filamentous ---6

5b. Alga unicellular or colonial [unusual in lotic periphyton and not considered in this key; see Prescott (1962, 1964) for more details]

6a.   Large macroscopic "plantlike" alga often several decimeters long; branches whorled at nodes ------------------------------- *Chara* or *Nitella* (Figure 7)

6b.   Alga usually much smaller and lacking whorled branches -------------------- 7

7a.   A true filament or pseudofilament; multicellular linear arrangement, branched or unbranched --- 8

7b.   A long branching unicellular tube without cross walls (coenocytic); cell wall may be occasionally constricted ------------------------------*Vaucheria* (Figure 8)

8a.   Filament branched ---9

8b.   Filament not branched --- 16

9a.   Growing on the back of a snapping turtle; branching only near the base -------- *Basicladia* (Figure 9)

9b.   Not growing on the back of a snapping turtle ---------------------------- 10

10a.   Short prostrate filament growing epiphytically on a larger filament; bristle-like setae arising from some of the cells ------------------ *Aphanochaete* (Figure 10)

10b.   Not as above --- 11

11a.   Filament in tough mucilage, often forming macroscopic hemispherical green growths difficult to mash in wet mount; tips of filaments tapered to points or hairlike bristles ------------------------------------*Chaetophora* (Figure 11)

11b.   Not in tough mucilage --- 12

12a.   Some cells bearing bristles, setae, or filaments tapering to fine points -------- 13

12b.   No setae present; ends of filaments rounded ----------------------------- 15

13a.   Setae with large bulbous bases -------------------------*Bulbochaete* (Figure 12)

13b.   Filaments without bulbed setae -- 14

14a.   All cells of the filament about the same diameter, highly branched ------------- *Stigeoclonium* (Figure 13)

14b.   Central axis of large cylindrical cells with cells of branches much smaller in diameter --- *Draparnaldia* (Figure 14)

15a.   Sparsely branched cells many times longer than broad ------------ *Rhizoclonium* (Figure 15)

15b.   Profusely branched and bushy; often covered with epiphytes -------- *Cladophora* (Figure 16)

16a. Cells slightly or grossly constricted in the middle -------------------------17

16b. Cells of filament not constricted -- 18

17a. Constriction very slight; cylindrical cell often with broad sheath ----- *Hyalotheca* (Figure 17)

17b. Constriction deeper or cells not cylindrical --------*Desmidium, Spondylosium,* or *Bambusina* (Figures 18–20)

18a. H-pieces present (filaments fragment in the center of cells rather than between cells) ----------------------------- *Tribonema* or *Microspora* (Figures 21–22)

18b. H-pieces absent --19

19a. Chloroplast parietal, with a large water-filled vacuole in the center of the cell -- 20

19b. Chloroplast axial; a plate or star-shaped ---------------------------------- 22

20a. Chloroplast a parietal spiral ----------------------------- *Spirogyra* (Figure 23)

20b. Chloroplast not a parietal spiral --21

21a. Chloroplast a parietal reticulum, cells sometimes slightly club-shaped ---------- *Oedogonium* (Figure 24)

21b. Chloroplast a parietal bracelet -------------------------- *Ulothrix* (Figure 25)

22a. Chloroplast an axial plate ----------------------------- *Mougeotia* (Figure 26)

22b. Two axial star-shaped chloroplasts per cell ----------------*Zygnema* (Figure 27)

23a. Alga filamentous; chloroplasts pink, maroon, or sometimes olive-green --------- Red Algae ------------------- *Audouinella* or *Batrachospermum* (Figures 28–29)

23b. Alga filamentous, colonial, or single-celled; chloroplast yellow-green, gold, or brown -----------------------probably a Diatom -----------------------24

24a. Alga single-celled or colonial --26

24b. Alga filamentous, cells arranged end to end ------------------------------25

25a. Cells boxlike and touching to form filaments or ribbons --------------------- *Fragilaria*, *Aulacoseira*, or *Melosira* (Figures 30–32)

25b. Cells more loosely arranged; alga usually in mountain streams -------- *Hydrurus* (Figure 33)

26a.   Cells usually in a colony or mucilaginous tube -------------------------- 27

26b.   Cells usually single or in groups at the end of stalks ---------------------- 29

27a.   Colony in the form of a zigzag chain ------- *Tabellaria*, *Diatoma* (Figures 34–35)

27b.   Colony otherwise; arranged in a tube or fan-shaped ---------------------- 28

28a.   Colony "fan-shaped" or sometimes forming a tight circle of cells; common in cold water --- *Meridion* (Figure 36)

28b.   Colony not fan-shaped; cells aggregated within a tube of mucilage ------------- *Encyonema*, *Nitzschia,* or *Navicula* (Figures 37–39)

29a.   Cells attached on one end by a stalk or pad; may occur singly or in groups --- 30

29b.   Cells not attached at one end -- 32

30a.   Cells wedge-shaped or club-shaped (one end wider than the other) ----------- *Gomphonema*, *Gomphoneis*, *Rhoicosphenia,* or *Meridion* (Figures 40–43)

30b.   Cells rectangular or bent in girdle view (both ends of equal width) ---------- 31

31a.   Cell rectangular in girdle view; often long and narrow ------ *Synedra* (Figure 44)

31b.   Cell a bent rectangle in girdle view; occurring most often in turbulent water --- *Achnanthidium* (Figure 45)

The remainder of the key beginning at couplet 32 requires that you observe frustular details from permanently mounted specimens employing an oil immersion objective at 1000X magnification. It is recommended that cleaned and mounted specimens be observed.

32a.   Cells circular in outline -------------------*Stephanocyclus*, *Discostella (*both are called *Cyclotella* in older publications), or *Stephanodiscus* (Figures 46–47)

32b.   Cells not circular in outline --- 33

33a.   Cells symmetric to both apical and transapical axes ----------------------- 38

33b.   Cells asymmetric to one or both axes ----------------------------------- 34

34a.   Cells sigmoid (S-shaped) --------------- *Gyrosigma* or *Nitzschia* (Figures 48–49)

34b.   Cells not sigmoid; either lunate or club-shaped --------------------------- 35

35a.   Cells club-shaped -- 37

35b.   Cells lunate --- 36

36a.   Cells with a clear axial area containing the raphe ----------*Cymbella*, *Amphora*, or *Reimeria* (Figures 50–52)

36b.   Cells lacking a clear axial area containing the raphe *Eunotia*, *Hannaea*, *Epithemia*, or *Rhopalodia* (Figures 53–56)

37a.   Cells with a raphe on one or both valves -------*Rhoicosphenia*, *Gomphonema*, or *Gomphoneis* (Figures 40–42)

37b.   Cells lacking a raphe ------------------ *Meridion* or *Martyana* (Figures 43, 57)

38a.   Cells with a raphe in the center of both valves; many genera fit this description including the common stream genera --------------------*Navicula*, *Stauroneis*, *Neidium*, *Sellaphora*, *Pinnularia*, *Craticula*, *Anomoeoneis*, *Brachysira*, and *Diploneis* (Figures 58–66)

38b.   Cells with a raphe lacking on one or both valves or with raphe along the margin of the cell -- 39

39a.   Raphe present on one valve only; cells often tightly attached to the substratum *Cocconeis* and *Achnanthidium* (Figures 67–68)

39b.   Raphe lacking or if present difficult to see along the margin of the valve ------ 40

40a.   Raphe lacking ----------------*Tabellaria*, *Diatoma*, or *Synedra* (Figures 69–71)

40b.   Raphe present along the margin of the cell and difficult to see ----------------- *Surirella*, *Nitzschia*, and *Cymatopleura* (Figures 72–74)

1a

1b

2a

2b

2c

2d

2e

2f

2g

2h

3

4a

4b

4c

4d
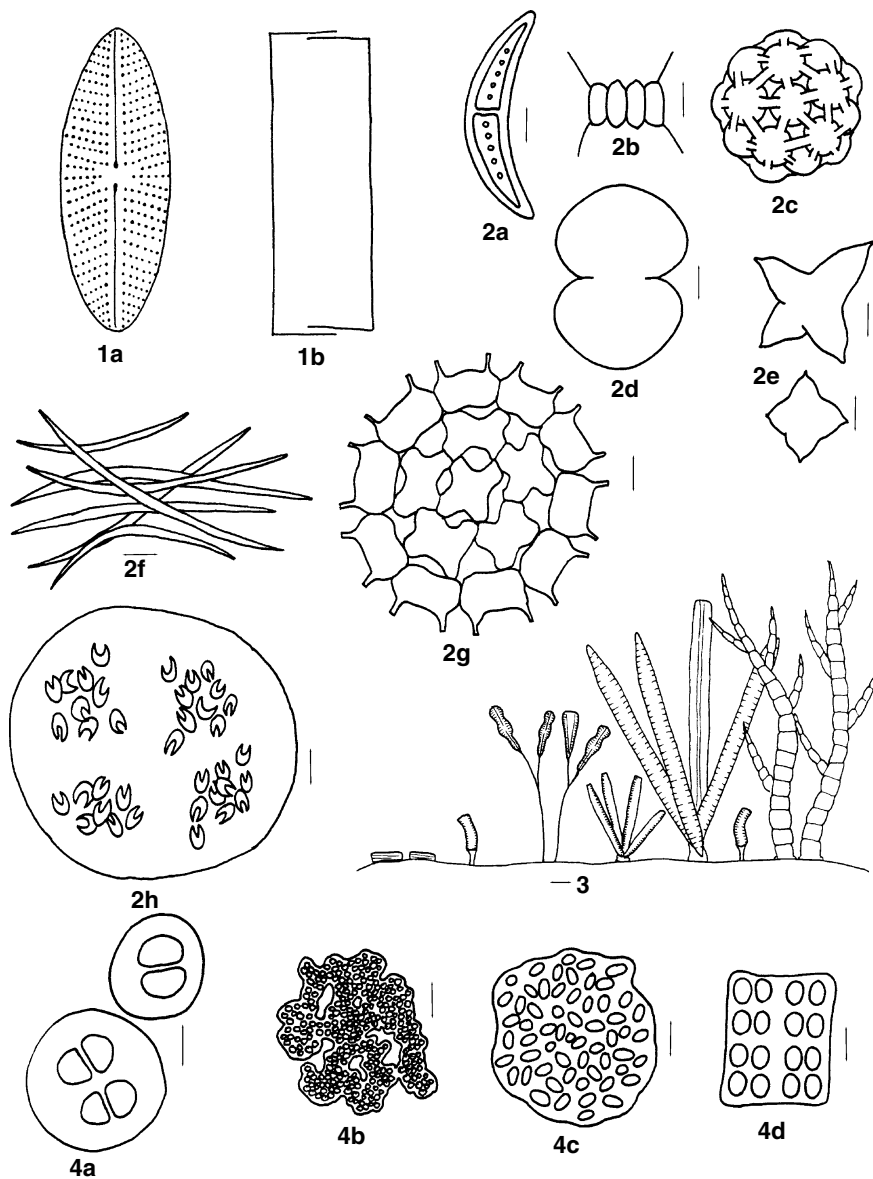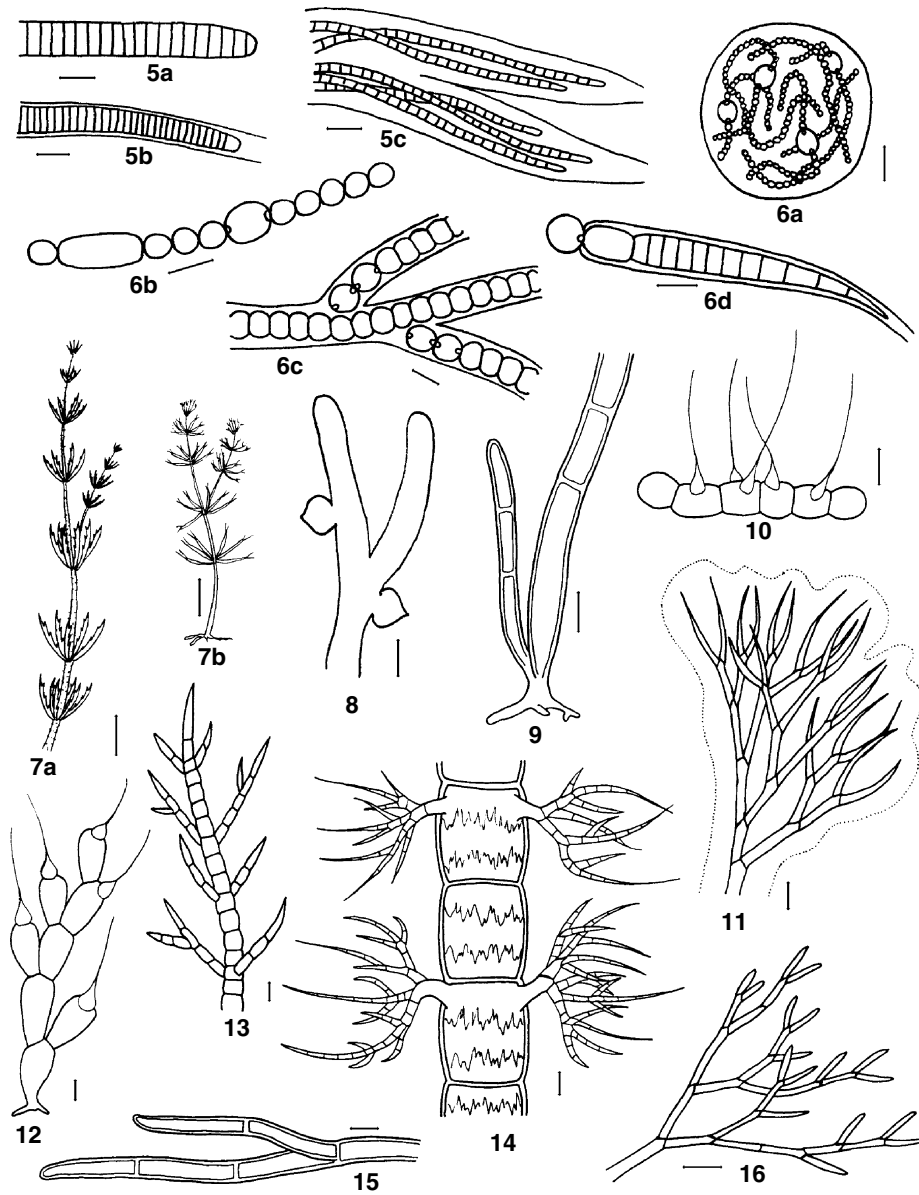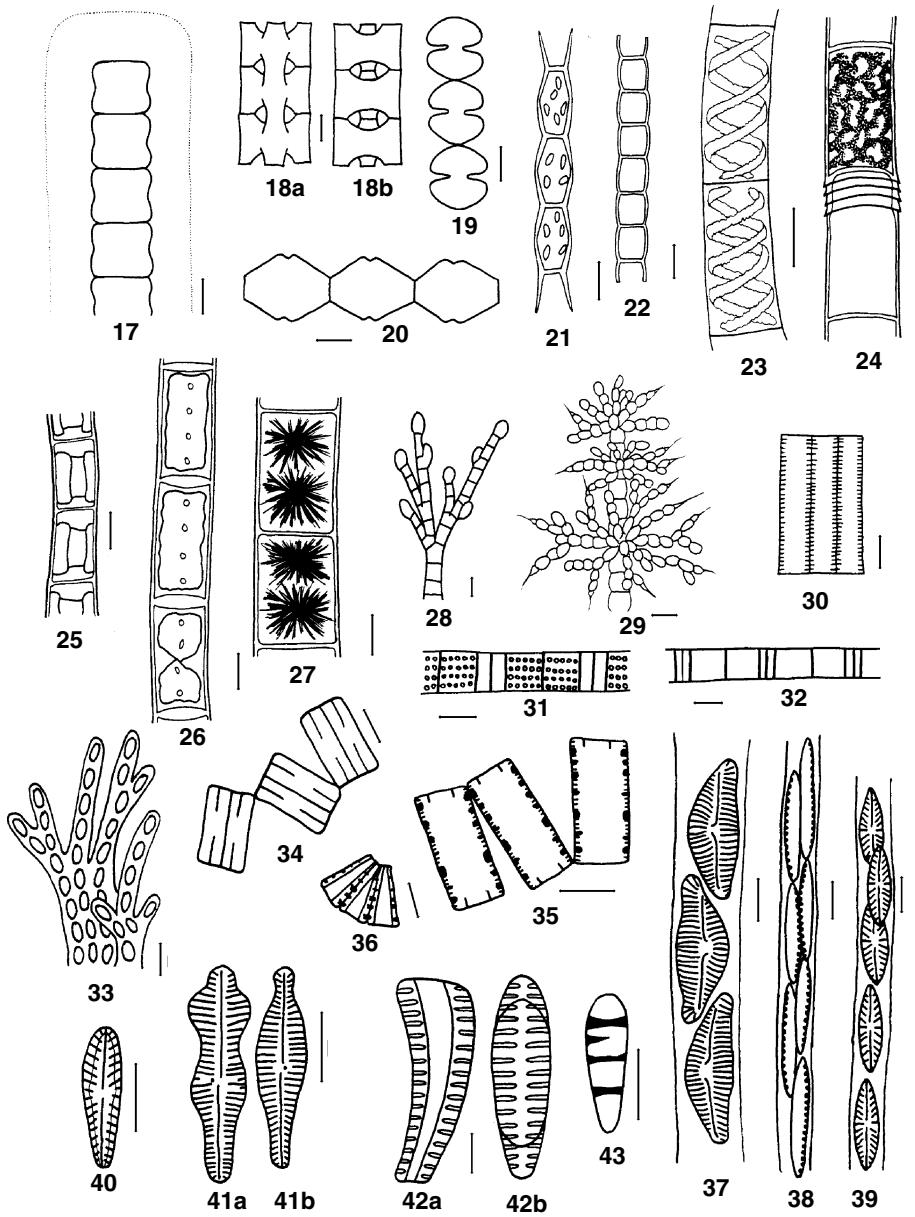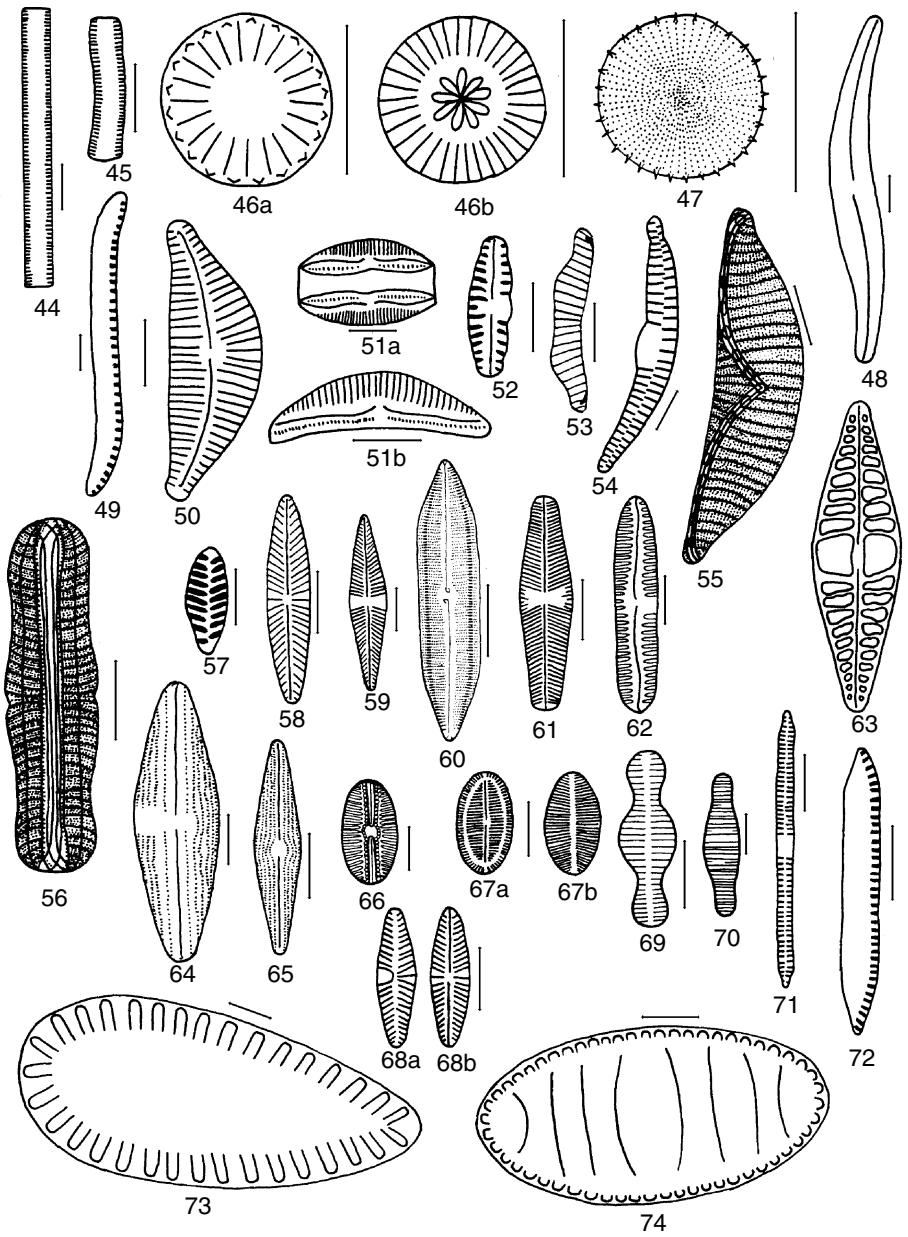
APPENDIX 16.3 LEGENDS: Figures of Benthic Algal Genera (All scale bars = 10 μm unless labeled otherwise)

Figure 1. Diatom frustule in a. valve view and b. girdle view. Figure 2. Non-filamentous green algae usually uncommon in stream periphyton: a. *Closterium* (scale bar = 25 μm), b. *Scenedesmus*, c. *Coelastrum*, d. *Cosmarium,* e. *Tetraedron*, f. *Ankistrodesmus*, g. *Pediastrum*, h. *Kirchneriella*. Figure 3. Diagram of periphyton physiognomy with diatoms (left) and a filamentous green alga (extreme right). Figure 4. Coccoid blue-green algae: a. *Chroococcus*, b. *Microcystis*, c. *Aphanothece*, d. *Merismopedia*. Figure 5. Filamentous blue-green algae lacking specialized cells: a. *Oscillatoria*, b. *Lyngbya*, c. *Schizothrix*. Figure 6. Filamentous blue-green algae with specialized cells: a. *Nostoc,* b. *Anabaena*, c. *Tolypothrix*, d. *Calothrix.* Figure 7. Macroscopic "plant-like" green algae: a. *Chara*, b. *Nitella*. Scale bar = 1 cm. Figure 8. *Vaucheria*, a siphonaceous green Chrysophyte. Scale bar = 100 μm. Figure 9. *Basicladia.* Figure 10. *Aphanochaete.* Figure 11. *Chaetophora.* Figure 12. *Bulbochaete.* Figure 13. *Stigeoclonium.* Figure 14. *Draparnaldia.* Figure 15. *Rhizoclonium.* Figure 16. *Cladophora.* Figure 17. *Hyalotheca.* Figure 18. *Desmidium* from a. front and b. back views. Figure 19. *Spondylosium.* Figure 20. *Bambusina.* Figure 21. *Tribonema.* Figure 22. *Microspora.* Figure 23. *Spirogyra.* Figure 24. *Oedogonium*, details of parietal reticulate chloroplast illustrated in upper cell; cells separated by apical caps. Figure 25. *Ulothrix.* Figure 26. *Mougeotia.* Figure 27. *Zygnema.* Figure 28. *Audouinella.* Figure 29. *Batrachospermum.* Figure 30. *Fragilaria.* Figure 31. *Aulacoseira.* Figure 32. *Melosira.* Figure 33. *Hydrurus.* Figure 34. *Tabellaria* colony. Figure 35. *Diatoma* colony. Figure 36. *Meridion* colony. Figure 37. *Encyonema* colony. Figure 38. *Nitzschia* colony. Figure 39. *Navicula* colony. Figure 40. *Gomphoneis.* Figure 41. *Gomphonema*: a. *G. acuminatum*, b. *G. parvulum*. Figure 42. *Rhoicosphenia*: a. girdle

view and b. valve view. Figure 43. *Meridion*, valve view. Figure 44. *Synedra*, girdle view. Figure 45. *Achnanthidium*, girdle view. Figure 46. a. *Stephanocyclus*, b. *Discostella.* Figure 47. *Stephanodiscus.* Figure 48. *Gyrosigma.* Figure 49. *Nitzschia.* Figure 50. *Cymbella.* Figure 51. *Amphora*: a. girdle view and b. valve view. Figure 52. *Reimeria.* Figure 53. *Eunotia.* Figure 54. *Hannaea.* Figure 55. *Epithemia.* Figure 56. *Rhopalodia.* Figure 57. *Martyana.* Figure 58. *Navicula.* Figure 59. *Stauroneis.* Figure 60. *Neidium.* Figure 61. *Sellaphora.* Figure 62. *Pinnularia.* Figure 63. *Craticula.* Figure 64. *Anomoeoneis.* Figure 65. *Brachysira.* Figure 66. *Diploneis.* Figure 67. *Cocconeis*: a. raphe-valve and b. rapheless-valve. Figure 68. *Planothidium*: a. rapheless-valve and b. raphe-valve. Figure 69. *Tabellaria.* Figure 70. *Diatoma.* Figure 71. *Synedra.* Figure 72. *Nitzschia.* Figure 73. *Surirella.* Figure 74. *Cymatopleura.*

APPENDIX 16.4 Detailed Taxonomic References for the Identification of Benthic Stream Algae

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Biomass and Pigments of Benthic Algae

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I. INTRODUCTION

Determination of biomass is one of the most fundamental measurements made in ecology. Although it is a static index, it provides information on the relative importance of either a taxonomic unit within a community (which has implications for competitive interactions) or of a community within an ecosystem (which has implications for trophic level interactions and energy flow). The measurement of benthic algal biomass is complicated by the fact that benthic algae invariably coexist as part of a complex assemblage variously referred to as periphyton, biofilm, or the German term *Aufwuchs* (Hynes 1970). This assemblage consists of algae, bacteria, fungi, and microzoans that are held within a polysaccharide matrix secreted by the microorganisms themselves (Lock *et al.* 1984). Consequently, assessment of algal biomass in the benthic assemblage is confounded by the presence of other organic constituents. Numerous techniques have been developed to measure benthic algal biomass, some of which attempt to account for the presence of nonalgal components, and others that treat the assemblage as a whole. Our objectives in this chapter are to (1) provide a context for the study of benthic algal biomass; (2) discuss in detail some of the more commonly used approaches to measure benthic algal biomass; and (3) describe a field exercise to examine the influence of irradiance on algal biomass, whereby these approaches can be employed and compared with each other to assess their individual performance.

Context for the Study of Algal Biomass

Although the energy base for stream ecosystems often derives from allochthonous sources (Hynes 1975, Vannote *et al.* 1980), autotrophic production can contribute a substantial fraction of fixed carbon to many streams (Minshall 1978, Cushing and Wolf 1984). Indeed, even in streams commonly considered heterotrophic, algal biomass may play a critical role as a food resource for herbivores (Mayer and Likens 1987, Lamberti 1996, Steinman 1996). Low algal biomass does not necessarily mean that the algae present are unimportant. The food quality of algae often is much higher than that of detritus, thus representing a more nutritious food resource for invertebrates (Cummins and Klug 1979). Modeling (McIntire 1973) and empirical (Gregory 1980, Sumner and McIntire 1982) studies have both revealed that lotic algal assemblages can support grazer biomasses approximately 10–20 times greater than their own because of high algal turnover rates. Although algal biomass does provide an index of how much food is potentially available to herbivores in a system at a point in time, it provides little information on how large a herbivore population can be supported by that biomass unless combined with an estimate of biomass turnover rates.

Not all algal biomass is consumed by herbivores. Calculations by Lamberti *et al.* (1989) on the fate of algal biomass grown in laboratory streams indicated that when algae are grown under high light conditions, the majority of material is exported from the system and not consumed by herbivores. Thus, sloughed algal biomass also can serve as a source of detritus or be collected by filtering heterotrophs downstream.

Short-term changes in algal biomass can be used as an index of productivity in a system (e.g., Bothwell 1988). However, one must be careful to control for immigration and emigration processes, as well as losses from grazing, which could confound the biomass accrual rate. In general, this calculation must be done with young assemblages (this reduces the chance of major emigration events) and under controlled conditions if the production rate is to have rigor.

Algal biomass levels can change rapidly in streams because of (1) disturbance events such as floods (Steinman and McIntire 1990, Peterson 1996) or (2) growth responses to changing environmental conditions (Rosemond *et al.* 2000). Consequently, drawing conclusions about stream conditions based on isolated sampling events of algal biomass can be misleading. Understanding the relationship between algal biomass and the stream ecosystem is best achieved through a systematic sampling regime, where biomass is sampled from the same general location(s) on a weekly or biweekly basis throughout an entire year (or longer, if feasible). In this way, both seasonal factors and chance events can be accounted for, and a more representative picture of algal biomass dynamics will emerge. Often, environmental factors are measured simultaneously with biomass, and correlations will be calculated between biomass and factors such as nutrient concentration, discharge, current velocity, temperature, irradiance, or grazer density (Biggs 1996). Based on the correlation results, hypotheses can then be generated regarding what factor(s) control algal biomass within a stream or at a site, which then can be tested using controlled experiments (e.g., Steinman 1992, Rosemond 1993, Wellnitz and Ward 2000).

Different Approaches to Measure Biomass: General Overviews

Ash-Free Dry Mass. This gravimetric approach involves drying the collected samples to a constant weight, oxidizing them in a muffle furnace, and reweighing the oxidized samples. The loss in weight upon oxidation is referred to as ash-free dry mass (AFDM). This

method has the advantages of requiring only basic laboratory instrumentation and being relatively nonlabor intensive. However, it does not allow the investigator to distinguish algal material from other organic material (e.g., fungi, bacteria) in the sample, nor does it account for the physiological state of the organic material (i.e., it may be senescent). Further, drying by heat may volatilize certain organic compounds and carbonates, leading to an underestimation of true AFDM. If one is interested in estimating algal biomass alone, this method may prove unsatisfactory, especially if there is a large fraction of nonalgal organic material in the sample.

Pigment Analysis. Three different methods are available for measuring chlorophyll *a* (and other pigments) in benthic algae: spectrophotometry, fluorometry, and high-performance liquid chromatography (HPLC). Spectrophotometry is perhaps the most common method used to measure algal pigments in streams; instrumentation is relatively common and inexpensive, and the protocols are straightforward. Fluorometry is more sensitive than spectrophotometry, requires less material, and can be used for *in vivo* measurements (Lorenzen 1966), which makes it ideal for pigment analysis of phytoplankton but usually unnecessary for benthic algae. HPLC is a very sensitive method that can measure a wide spectrum of accessory pigments as well as chlorophyll degradation products. However, the instrumentation is expensive and it is not standard equipment in many ecology laboratories. In this chapter we focus on spectrophotometry and HPLC. Although fluorometry is more sensitive than spectrophotometry, the relatively high algal biomass levels in streams can offset this advantage (i.e., that level of sensitivity is rarely needed). In addition, fluorometers must be calibrated against spectrophotometric standards.

Chlorophyll *a* is the most abundant pigment in plants (although not always in algae), and consequently its absorbance is measured most frequently. However, it is also possible to measure the absorbance of chlorophylls *b* and *c*, other accessory pigments, and the degradation products of these pigments. The main advantages of pigment analysis are (1) its relative simplicity; (2) its ability to differentiate algal biomass from non-autotrophic organic constituents in the assemblage; and (3) its ability to provide information on algal community structure if an entire suite of pigments is analyzed. Its main disadvantages include (1) the need for a spectrophotometer, fluorometer, or HPLC; (2) the amount of chlorophyll in an algal cell can change depending on species composition and environmental conditions; (3) extract concentration can change depending on solvent type, extraction method, and analytical procedure; and (4) samples may contain degradation products derived from allochthonous sources.

Biovolume. Measurement of individual algal cell volume provides a direct, but labor intensive, method for estimating benthic algal biomass. The methodology involves examination of cells under a light microscope, measuring cell dimensions using an ocular micrometer, and calculating cell volumes for each species by applying appropriate geometric formulae to each form (e.g., Wetzel and Likens 1991, Kirschtel 1996; see also http://diatom.acnatsci.org/nawqa/2001biovol.asp). This measurement can be included with the measurement of algal community composition, thereby providing information on both algal taxonomic structure and biomass simultaneously. One of the main advantages of this combined approach is its ability to assess the biotic impacts of toxic chemicals or other environmental stressors, which may restructure the community but have no net impact on biomass (Schindler 1987). However, biovolume measurement suffers from the difficulty in distinguishing between live and dead cells when cell counts are being made

using a microscope. Usually, only those cells with intact plastids are considered "live." The protocol associated with this procedure is covered in Chapter 16.

Overview of Chapter

This chapter compares algal biomass on substrata in stream reaches that are open to sunlight (high light) or are shaded by a riparian canopy (low light). Irradiance can profoundly affect algal biomass, productivity, and community composition in stream ecosystems. Both biomass and productivity are positively related to irradiance (Steinman and McIntire 1987, Steinman 1992, Hill *et al.* 2001, Hill and Dimick 2002), at least up until light levels result in photoinhibition (Hill 1996). Algal taxonomic structure also changes with light level (Sheath *et al.* 1986, Steinman *et al.* 1987, DeNicola *et al.* 1992, Wellnitz *et al.* 1996), although the effect is complex and varies with season, substrate type, current velocity, nutrient level, and herbivory.

The influence of light on algal communities can be profound in streams. Riparian canopies can intercept over 95% of ambient light, resulting in photosynthetically active radiation (PAR) levels that constrain algal growth (Steinman 1992, Hill *et al.* 2001). This constraint has ecosystem implications, as reduced autotrophic growth can result in greater streamwater nutrient concentrations (Hill *et al.* 2001) due to less biotic uptake, as well as reduced secondary production because of food limitation (Hill *et al.* 1995, 2001). This change in light level is particularly important in deciduous forest streams, where leaf emergence and abscission result in dramatic changes in PAR over relatively short time periods (Hill and Dimick 2002). However, even when leaves are fully developed, many streams have patches of open and shaded reaches because of natural causes or anthropogenic disturbances. In this chapter, we focus on the influence of PAR on algal biomass in open and shaded reaches. As you evaluate these different reaches, consider what other factors are influenced by light, the effect of time of day on measuring light, and how time of year may affect your measurements. Predict where and when you think algal biomass will be greatest before starting this exercise.

Two specific questions will be addressed in this chapter. First, does benthic algal biomass differ between shaded and open stream reaches? Second, do different approaches for measuring benthic algal biomass produce consistent results? In addition, optional methods allow researchers to determine if benthic algal biomass differs between natural and artificial substrata and whether algal biomass is correlated with specific environmental parameters. Because the drying and extraction times involved in the measurement of biomass and pigments are relatively long, we have designed the laboratory portion of the chapter to be completed in two separate sessions.

II. GENERAL DESIGN

A. Overall Design of Methods

The methods presented herein are designed to examine potential differences in algal biomass between high-light and low-light stream reaches and to examine potential differences in methods of measuring benthic algal biomass (AFDM vs. pigment analysis). Researchers sample paired high-light/low-light stream reaches, collect artificial substrata from each reach, and analyze AFDM and chlorophyll *a* from each substratum type. See Chapter 5 for additional information on light and its measurement in streams.

B. Site Selection

Ideally, the stream selected will have open and shaded reaches. However, if the streams in your region lack overhanging riparian vegetation (e.g., many desert, prairie, or alpine streams), an artificial canopy can be created by suspending greenhouse shade cloth over the stream to simulate shade (cf. Hepinstall and Fuller 1994) or sites under bridges can be contrasted to adjacent reaches in the open, to provide a low-light/high-light contrast. Alternatively, if the streams are entirely shaded, it may be possible to suspend lights across the stream to increase irradiance (cf. Steinman 1992). In general, wadeable second- or third-order streams are ideal for the methods given here.

C. Overview of Analytical Procedures

Substratum Type. Natural substrata vary in size, texture, and origin, thereby introducing an element of variation into the sampling process. As an alternative, investigators sometimes use artificial substrata of known size and texture to reduce substratum variability. Historically, glass slides were used as artificial substrata (e.g., Ivlev 1933, Patrick *et al.* 1954), but some studies have shown that algal communities grown on glass slides do not accurately represent natural communities (Brown 1976, Tuchman and Blinn 1979). As a consequence, other substratum types have been employed. Both Tuchman and Stevenson (1980) and Lamberti and Resh (1985) reported that clay tiles produced more reproducible results than sterilized rocks. In this chapter, benthic algae will be sampled from artificial (clay tile) substrata. In an optional method, comparisons of biomass can be made between natural and artificial substrata.

Collection of Algae. The type of device used to remove algae from substrata for biomass determination will depend on the location, size, and texture of the substratum being sampled. Many different types of devices have been designed for collecting benthic algae (see reviews by Sladeckova 1962 and Aloi 1990). In general, if the substratum is too large or too deep to move, then a sampling device similar to, or modified from, the one suggested by Douglas (1958) can be utilized. Recent advances have resulted in a plexiglass chamber or tube, which is placed over the portion of the substratum that is to be sampled (Figure 17.1). A neoprene (rubber) gasket at the base of the chamber improves the seal between the chamber and the substratum, preventing leakage of scraped material. A sharp blade or brush is inserted into the chamber and used to remove attached algae from the substratum. A syringe is then either placed inside or attached to the chamber and the scraped slurry is sucked out (e.g., Loeb 1981).

In situations where the substratum can be removed from the stream, there is no concern that scraped material could be washed downstream. In this case, the entire exposed surface or a specific area of the substratum (Figure 17.1) can be scraped or brushed. In the case of pigments, the entire substratum (if small enough) can be placed in a container with solvent for pigment extraction (although this method does create more solvent waste than extracting pigments from scraped material). For substrata with substantial algal biomass, it is best to remove material using the syringe sampler described above or with a razor blade. If using the razor, the attached algae should be removed over a small pan, plastic container, or into a plastic bag, so that none of the loose material is lost. After scraping, the remaining material can be removed by vigorous brushing with a hard-bristled toothbrush. It is important to clean any brush thoroughly before it is used again on a different substratum. For substrata without much algal biomass, removal can
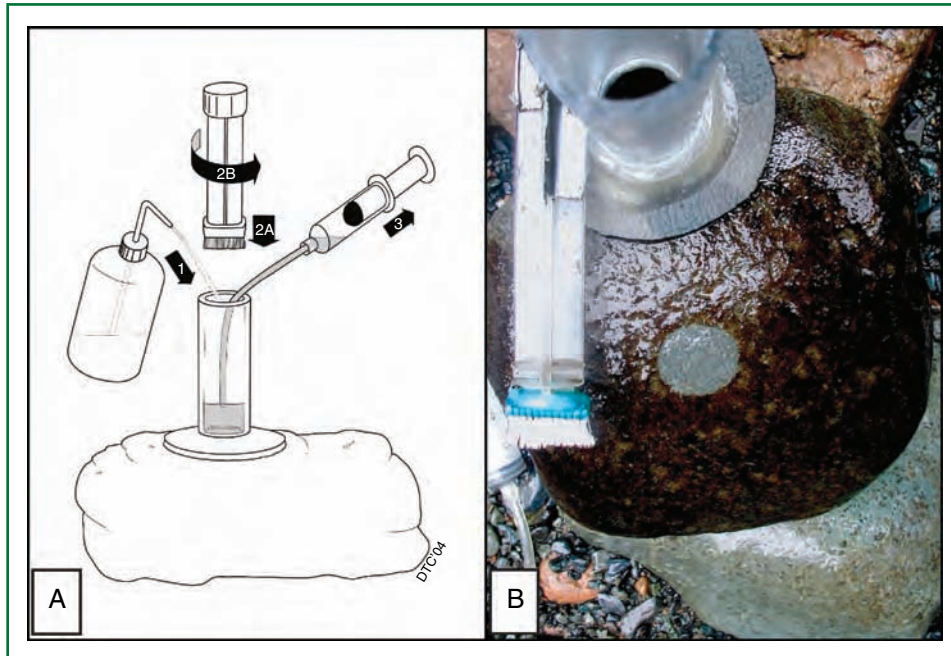
FIGURE 17.1 Tubular sampler suitable for removing periphyton from a known area of stream substratum. Sampler consists of open-ended plexiglass tube with basal neoprene seal (modified from Loeb 1981). (A) Sampler is manually held firmly to rock surface and streamwater is squirted into the chamber (step 1), a modified syringe plunger with toothbrush head is then inserted into the chamber (step 2A) and rotated to remove periphyton (step 2B), and the algal slurry is pulled from the chamber with a syringe extended with tygon tubing (step 3). This procedure is repeated two to three times to ensure that all periphyton is removed. (B) Sampling "divot" on rock surface resulting from periphyton removal by the tubular sampler. (Illustration by D. Chaloner; photo by G. Lamberti.)

begin with the brush. However, even vigorous brushing is not 100% effective in removing algal biomass (Cattaneo and Roberge 1991). Once a slurry is collected, it should either be filtered in the field or transported back to the laboratory for filtration and processing (see *Basic Method 1* below for details on filtration). Either way, the sample should be placed on ice and in the dark as soon as possible after collection.

Dry Mass and Ash-Free Dry Mass. After filtration, the preweighed filter is placed into a numbered (to keep track of each sample) aluminum weigh boat or porcelain crucible and dried to constant weight at 105°C (usually for 24 hr). The dried material is weighed to the nearest 0.1 mg, oxidized at 500°C, and reweighed. Some researchers recommend re-wetting the oxidized material with deionized water and re-drying to constant weight at 105° C. This will reintroduce the water of hydration in clay, which is not lost at 105°C, but is volatilized at 500°C (Nelson and Scott 1962). It has been our experience that this loss is extremely low (<1% of AFDM) and can be ignored for most purposes, but if clay is very abundant in the stream, it may be worthwhile to conduct some pilot studies to determine whether rewetting is necessary. Dry mass (DM) is calculated as the weight of the dried material plus filter (usually in mg) minus the original filter weight, divided by the area of the sampled substratum (usually in cm^2). AFDM is calculated as the weight

of the DM minus the residual ash, divided by the area of the sampled substratum (see *Data Analysis* sections for details).

Chlorophyll and Degradation Products. Living algae contain mainly undegraded chlorophyll molecules (e.g., Chl *a* alone or together with Chl *b* or Chl *c*). However, as a consequence of cell senescence, death, or the presence of detritus, samples also may include chlorophyll degradation products. The two most common degradation products of chlorophyll *a* are pheophytin *a* and pheophorbide *a*. All chlorophylls contain a central magnesium ion, which is bonded to four nitrogen atoms in a ring structure (Figure 17.2). The Mg^{2+} is lost if chlorophyll is exposed to an acidic environment. Pheophytins are created when the magnesium is lost from the structure. Alternatively, the loss of the phytol tail from ring D (Figure 17.2) results in a molecule termed a chlorophyllide. Further degradation of either the pheophytin (due to the loss of phytol) or the chlorophyllide (due to the loss of Mg^{2+}) produces a pheophorbide. Because the above degradation products have been reported to contribute up to 60% of the measured chlorophyll *a* content in fresh water (Marker *et al.* 1980) and can absorb light in the same region of the spectrum as does chlorophyll *a*, their presence can interfere with the spectrophotometric estimation of chlorophyll *a* concentration. Thus, estimation of chlorophyll *a* requires absorbance to be measured both prior to, and following, acidification, in order to correct for pheophytin that may have been present. An alternative spectrophotometric approach is simply to measure total chlorophyll pigments (Golterman and Clymo 1971), which estimates all chlorophyll pigments and degradation products that absorb at 665 nm.

After filtration of the sample, the algae can either be extracted immediately or frozen (in the dark) for analysis the following week. Physical disruption of the assemblage with a tissue grinder, sonicator, or by deep freezing at −20°C is often recommended
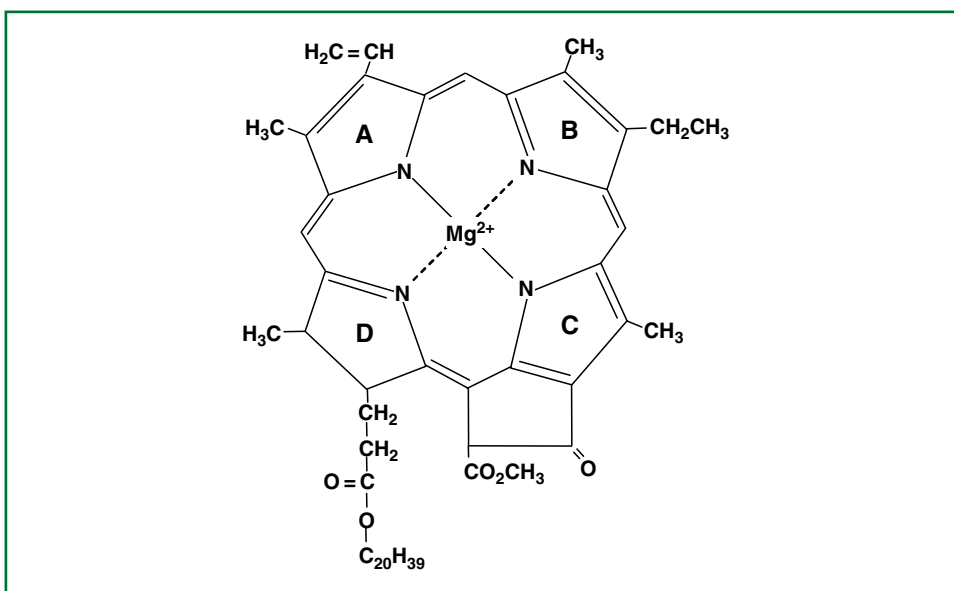
FIGURE 17.2 Structure of chlorophyll *a*, showing the central core of magnesium held in a porphyrin ring. Chlorophyll *b* is identical to chlorophyll *a* except for the substitution of a −CHO group in place of the −CH₃ group in ring B.

to facilitate extraction, but Axler and Owen (1994) found no statistically significant difference in chlorophyll *a* content of phytoplankton extracted with and without grinding. However, assemblages dominated by cyanobacteria may require grinding (Marker *et al.* 1980). For the purposes of the methods described here, we do not recommend grinding unless cyanobacteria are the dominant algal class present in the sample; the potential disadvantages of filters sticking to the bottom of grinding tubes and potential loss of sample during processing argue against grinding under most circumstances (Axler and Owen 1994). Instead, we recommend that samples be frozen at −20°C or colder for one to two hours prior to extraction with organic solvents. Here, the sample is thawed and is allowed to steep in solvent for a minimum of one to two hours, before being centrifuged gently (if needed) to separate the supernatant (containing chlorophyll) from the pellet (filter plus disrupted organic material). The absorbance of a known volume of supernatant is read on a spectrophotometer (see Section III for details).

Chlorophyll and carotenoid pigments can also be quantified using reversed-phase high performance liquid chromatography (RP-HPLC). RP-HPLC differs from spectrophotometric analysis because it relies on the separation of pigments based on their chemical structure and polarity. Chromatographic separation involves the differential attraction of pigments to packing material in a column (stationary phase) and the solvent stream (mobile phase) moving through the column (Leavitt and Hodgson 2001). The stationary phase is usually nonpolar (lipid-soluble), whereas the mobile phases are variably polar (composed of several organic solvents, such as methanol and acetone); as a consequence, polar pigments move through the column relatively fast and are detected first. A wealth of literature covers the criteria and guidelines for choice of HPLC instrumentation and protocols (e.g., Pfander and Riesen 1995, Wright and Mantoura 1997, Jeffrey *et al.* 1999), so we refer readers to those papers for additional detail. In this chapter, we cover the basic principles and approaches of HPLC.

D. Optional Approaches

Two optional approaches are suggested for additional insight. The first option involves comparing biomass quantity on natural versus artificial substrata. In these methods, researchers collect artificial and natural substrata in each open/shaded habitat and analyze AFDM and chlorophyll *a* on both substratum types. Student *t*-tests are conducted to detect statistical differences between artificial and natural substrata for AFDM and chlorophyll in open and shaded reaches. The second option is to measure the environmental conditions in the two habitats (e.g., current velocity, discharge, nutrient concentration, grazer density) and correlate these data with the biomass data. It is important to remember that a statistically significant correlation does not prove causation but rather provides a basis for generating hypotheses about possible mechanisms controlling benthic algal biomass, which could then be tested with experiments.

E. Data Analysis

The AFDM and pigment data will be normalized per unit area sampled. The following *t*-tests will be performed:

1. AFDM in open reach vs. AFDM in shaded reach
2a. Chlorophyll *a* in open reach vs. chlorophyll *a* in shaded reach (spectrophotometry)
2b. Pigments in open reach vs. pigments in shaded reach (HPLC)

In addition, an autotrophic index (AI) will be calculated. This index (AFDM/chlorophyll *a*) provides information on the trophic status or relative viability of the periphyton community. If large amounts of non-living organic material are present, the numerator becomes inflated, and the ratio exceeds the normal range of 50–200 (APHA *et al.* 1995).

III. SPECIFIC METHODS

A. Basic Method 1: Open vs. Shaded Reach Comparison Using Artificial Substrata

Preparation Protocol

1. At least one month prior to the exercise, and preferably several months in advance, place unglazed ceramic tiles in selected open and shaded reaches in the stream to be sampled. If the tiles were purchased in attached sheets, as opposed to separate units, place the entire sheet in the stream, which reduces the likelihood of the tiles being displaced by floods. It is best to place the tiles in riffles that contain similarly sized natural substrates to minimize the chance of burial by fine sediment. Each team will analyze 8 tiles per site (4 for AFDM; 4 for pigments), for a total of 16 tiles for each open/shaded pair of sites (8 for AFDM; 8 for pigments). Therefore, each team member will sample at least one tile for AFDM and one tile for pigments in the open and shaded reaches. (If the number of researchers is limited, each team member may have to sample more than one tile for AFDM and pigments per reach type). Note that if individual tiles are used (i.e., not sheets of tiles), set out twice the anticipated number of tiles to be used in the exercise, as floods may result in the loss of tiles during the colonization period.

2a. If tile sheets or natural substrates are collected: Label plastic food containers (ca. 900 cm^2) according to team ID and open or shaded site number (e.g., Team A-Open1).

2b. If individual tiles are collected: Each team should obtain appropriate sample containers (use black film canisters or 20-mL scintillation vials labeled according to team ID, open or shaded site number, AFDM or chlorophyll, and tile number (e.g., TeamA-Open1-AFDM1).

3. Weigh at least 100 precombusted glass fiber filters to the nearest 0.1 mg and place them in appropriately numbered containers (aluminum weigh boats with a number etched on their bottom with a sharp instrument work well). Either Whatman GF/C (1.2-μm pore size) or GF/F (0.7 μm pore size) filters, or their equivalent, are adequate for the algae found in most streams (Prepas *et al.* 1988). Filter diameter should match whatever filtering assembly is being used in the classroom or laboratory.

Protocol for Field Collection and PAR Measurements

1. Collect tiles from open and shaded reaches.

2a. Using plastic food containers: Fill two plastic containers (labeled as described above) with stream water. Place 10 tiles (2 extra, to accommodate mishandling or unanticipated problems) from each habitat inside the container. Attach the lids, squeeze out excess water (keeping the containers filled minimizes tile movement), and place the containers in coolers to be transported back to the laboratory.

2b.   Using film canisters or scintillation vials: Place one tile in each holder (labeled as described above) with stream water. Place 10 tiles (2 extra, to accommodate mishandling or unanticipated problems) from each habitat inside the container. Attach the lids, squeeze out excess water (keeping the containers filled minimizes tile movement), and place the containers in coolers to be transported back to the laboratory.

3.   Estimate irradiance levels in the open and shaded sites. Use protocols for determination of irradiance provided in Chapter 5.

Laboratory Protocol: Biomass Removal and Pigment Extraction and Filtration

1.   In the laboratory, separate the sheet of tiles (if appropriate) into individual tiles (ignore any glue that may remain attached to individual tiles following separation).

2.   Using a hard-bristled toothbrush, brush off the algae on the tile, collecting the brushed material into a pan (or tray). Use a squirt bottle filled with distilled water to periodically wash the tile and toothbrush. Be conservative in the amount of water used. Carefully pour the removed material into appropriately labeled vials.

3.   Filter each slurry onto a glass fiber filter (precombusted and preweighed in the case of biomass) using a standard filtration apparatus and no more than 15 psi of vacuum (to avoid rupturing of cells). For pigment analysis, filters can be placed in labeled opaque containers (e.g., aluminum foil or film canisters) and frozen until the following laboratory session.

4a.   AFDM: Put the filter back into the weigh boat and place inside a drying oven set at 105°C (APHA *et al.* 1995). Generally, it will take at least 24 hr for the biomass to reach constant mass. Consequently, the remainder of the AFDM determination will be done in the second laboratory session. To facilitate finishing the exercise, we recommend that a researcher or team leader remove the weigh boats from the drying oven during the week and transfer them to desiccators.

4b1. Chlorophyll *a* by spectrophotometry: After filtration, place the filter into a small container (e.g., black film canister) or centrifuge tube containing a known volume (just enough to cover filter) of 90% buffered acetone (90 parts acetone and 10 parts saturated magnesium carbonate solution[1,2]). Extract samples for at least 2 hr, and preferably 24 hr, at 4°C (or on ice) in the dark.[3]

4b2. *Advanced Method 1: Chlorophyll a by HPLC:* After extraction in acetone (see above), filter the extract through a 0.2 μm pore-size, chemically resistant filter to remove small particles. Transfer 2.0 mL of extract to a clean vial and dry completely under a stream of N_2 gas under low light. Drying should use a N_2 gas flow that is sufficient to "dimple" the surface of the extract. Evaporation of water can be enhanced by adding 0.5 mL of pure benzene[4] to the extract solution until

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[1] Other solvents (e.g., methanol, ethanol, DMSO) may be used to extract chlorophyll, but the equations given in this chapter are based on the use of acetone. Wear gloves, lab coat, and eye shields when working with acetone or other solvents.

[2] $MgCO_3$ helps minimize degradation of chlorophylls, but some studies have found that its addition has no significant effect (Lenz and Fritsch 1980) and may even absorb pigments (Daley *et al.* 1973).

[3] Chlorophyll is subject to photodegradation. Extraction of chlorophyll must be done in dim light or dark.

[4] Extreme caution should be taken in the use of benzene. Benzene is a known carcinogen and is highly flammable. The risks of using it in the laboratory must be fully assessed before work begins.

all solution is completely evaporated. Once dry, dilute samples to 400 μL with an injection solvent containing an internal standard. It should be noted that solvent choice may affect extraction efficiency (cf. Cartaxana and Brotas 2003).

Laboratory Protocol: AFDM Measurement

1. If the filters have not been removed from the drying ovens during the week, remove them and place them in desiccators at the start of the lab. Keep filters in the desiccator until they cool to room temperature.
2. Weigh the filter (use forceps to remove filter from weigh boat) on an analytical balance (to the nearest 0.1 mg).
3. After weighing the filter, return it to the aluminum weigh boat and oxidize the material at 500°C for 1 hr. Keep in mind that it may take an hour or two (depending on size and style) to bring the muffle furnace temperature to a constant 500°C.
4. Remove filters with oxidized material from the muffle furnace and allow them to cool in the desiccator.
5. Weigh the filter (use forceps to remove filter from weigh boat) on an analytical balance (to the nearest 0.1 mg).

Laboratory Protocol: Spectrophotometric Analysis of Chlorophyll *a*

1. Room lights should be dimmed during chlorophyll measurements to avoid changes in absorbance values. Remove the chlorophyll extract from ice, if necessary. Centrifuge the sample if grinding was employed.
2. Transfer 3 mL of extract to a 1-cm glass cuvette and read optical density (OD) at 750 and 664 nm. The absorption at 750 nm is subtracted from the reading at 664 nm to correct for the presence of turbidity and colored materials (Wetzel and Likens 1991).
3. Acidify the extract in the cuvette with 0.1 mL of 0.1N HCl. Gently agitate the acidified extract (lightly "flick" the cuvette with finger), wait 90 sec, and read OD at 750 and at 665 nm.
4. Rinse the cuvette with 90% acetone and shake dry prior to measurement of the next sample.

Laboratory Protocol for Advanced Method 1: HPLC Analysis of Pigments

1. Room lights should be dimmed during pigment measurements to avoid changes in absorbance values. Pigments should be dissolved in injection solvent as in Advanced Method 1 (above).
2. Make up HPLC solution A and B and transfer to HPLC solvent reservoirs. Run HPLC pumps on 50% solvent A and 50% solvent B until pressure is stable at ca. 3000 psi.
3. Transfer 400 μL of each extract to a separate HPLC sample vial. Transfer 400 μL of each pigment standard (e.g., beta-carotene, chlorophyll *a*) to separate sample vials. Clean syringe, pipettes, and filter with 2 mL of 100% acetone between each

transfer. Place standards into auto-sampler carousel at positions 1 and 2. Load sample extract vials at subsequent positions (3, 4, 5, etc.).

4. Enter sample sequence data into the HPLC computer program. Run HPLC pumps with 100% solvent A for 5 min. Inject 100–200 μL of sample to start analysis using the procedure of Leavitt and Hodgson (2001). Using Table 17.3, record time at which peaks are detected by monitoring detector absorbance between 300–800 nm (photodiode array detector) or at 435 nm (spectrophotometer). Each sample will take about 35 min to complete.

4a. Alternative (if no computer controller is available): Inject 100–200 μL of sample while running 100% solvent A at 1.5 mL min^{-1} (ca. 3500 psi). Follow timed changes in solvent composition:

> 0.0 min — inject sample, start peak area integration, run 100% solvent A.
> 2.5 min — change solvent composition to 80% solvent A.
> 3.5 min — change solvent composition to 65% solvent A.
> 4.5 min — change solvent composition to 45% solvent A.
> 5.5 min — change solvent composition to 25% solvent A.
> 6.5 min — change solvent composition to 10% solvent A.
> 7.0 min — change solvent composition to 0% solvent A.
> 32 min — end integration; change solvent composition to 25% solvent A.
> 33 min — change solvent composition to 50% solvent A.
> 34 min — change solvent composition to 75% solvent A.
> 35 min — change solvent composition to 100% solvent A and restart cycle
> (next sample injection in 5 min).

5. Record peak areas from digital integrator printout. Convert peak areas to mass of pigment injected using calibration curves for each standard pigment. These curves should be prepared in advance by injecting 5–10 samples of each pigment standard (e.g., 20, 40, 60, 80, 100 μL) and determining the slope of the relation between integrator peak area and mass of pigment injected. The pigment mass injected is calculated as pigment concentration in standard (in μg μL^{-1}) multiplied by μL injected. Assume all carotenoids (nonfluorescent pigments) have similar calibration curves to that of beta-carotene, and that chlorophylls and their derivatives (fluorescent pigments) are similar to chlorophyll *a* (note that because not all carotenoids will match beta-carotene, nor will all chlorophylls match chlorophyll *a*, this assumption will introduce some error into the calculation but we believe the error is small relative to the simplicity gained using this approach). Calculate total amount of each pigment in original extract and express as a function of substrate surface area.

B. Basic Method 2: Analysis of Pigments on Natural versus Artificial Substrata

This method allows the researcher to compare the effectiveness of ceramic tiles in representing natural substrata (cf. Lamberti and Resh 1985). It requires sterilization of cobble prior to the exercise.

Preparation Protocol

1. At least one month prior to the exercise, and preferably several months in advance, collect natural substrata (preferably small cobble with relatively flat surfaces) from

the stream. Scrub the substrata and either autoclave, ash in a muffle furnace, or immerse in acetone for 24 hr to remove and kill all attached organic material.
2. Place the sterilized rocks and unglazed clay tiles adjacent to each other in open and shaded reaches of the stream.

Field Collection

1. Collect four rocks and four tiles from each open and shaded reach and place them in plastic food containers as outlined previously. Instead of scraping the substrata, place them directly into separate plastic, wide mouth jars containing sufficient 90% acetone to cover the exposed surface of the substratum (approximately 30 mL).
2. When adding the substrata to the solvent, make certain that the upper surface of the substrata (the surface exposed to light in the stream) is placed face down in the jar (to ensure immersion of algae) and add them slowly, to minimize splashing and loss of solvent.

Laboratory Procedures

1. Following removal of the algae from the rock substrata, estimate rock surface area. We recommend use of the "aluminum foil method" (Lamberti *et al.* 1991). If the rocks have been placed in solvent to extract pigments, allow them to dry completely in a hood (place them on a labeled paper towel that corresponds to the sample number associated with the rock). After the rocks are dry, wrap each rock completely, avoiding overlap, in aluminum foil. Trim excess foil with scissors. Remove the foil wrap and weigh on a balance. Also, weigh an unfolded square of known area. The following equation is used to calculate the unknown rock area (A_r):

$$A_r = \left(\frac{A_k}{W_k}\right) \times W_{rf} \tag{17.1}$$

where A_k = known area, W_k = known weight, and W_{rf} = weight of rock foil. Make sure to keep units consistent (e.g., cm^2 for area; mg for mass). To estimate "colonized" surface area, A_r typically is divided by two (assume that only the top half of the rock is covered by periphyton).
2. Follow laboratory procedures above to measure chlorophyll *a* spectrophotometrically or pigments by HPLC.

C. Advanced Method 2: Correlating Biomass with Environmental Variables and Relating

Biomass to Algal Taxonomic Structure

1. At each site where algae are collected, measure irradiance as described in Chapter 5.
2. At each site where algae are collected, measure inorganic nitrogen and soluble reactive phosphorus as described in Chapters 8–10, or in Standard Methods (APHA *et al.* 1995).

3. At each site where algae are collected, measure densities of grazing invertebrates as described in Chapters 20 and 23.
4. Coordinate algal biomass collection with measurement of algal community structure (Chapter 16) to determine which taxa account for the majority of biovolume in each community.

D. Data Analysis

Dry Mass and AFDM[5]

1. Calculate the dry mass (DM, in mg/cm^2) and AFDM (in mg/cm^2) of the biomass on each tile (or rock):

$$DM = \frac{(W_a - W_f)}{A_{t/r}} \tag{17.2}$$

where W_a = dried algae on filter (mg), W_f = filter weight (mg), and $A_{t/r}$ = area of tile or rock (cm^2) (see Table 17.1); and

$$AFDM = \frac{(W_a - W_f) - W_{ash}}{A_{t/r}} \tag{17.3}$$

where W_{ash} = material on filter (mg) after ashing.

Major Pigments by Spectrophotometry

1. Calculate the chlorophyll *a* and pheophytin concentrations of the periphyton on each tile or rock (see Table 17.2) based on spectrophotometric analysis:

Chlorophyll *a* (μg/cm^2) = 26.7 (E$_{664b}$ − E$_{665a}$) × V$_{ext}$/area of substrate (cm^2) × L

$$\tag{17.4}$$

and

Pheophytin (μg/cm^2) = 26.7 (1.7E$_{665a}$ − E$_{664b}$) × V$_{ext}$/area of substrate (cm^2) × L

$$\tag{17.5}$$

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[5] Some researchers report AFDM as g/m^2 instead of mg/cm^2; to convert from mg/cm^2 to g/m^2, multiply by 10.

TABLE 17.1	Sample Data Sheet for Determining Dry Mass (DM) and Ash-Free Dry Mass (AFDM).

Stream: Date:

Location: Investigators:

Open/Shaded reach	Rep. #	Weigh boat #	A Filter mass (mg)	B DM + filter (mg)	C DM (mg) $= B - A$	D Ash + filter (mg)	E AFDM (mg) = $B - D$
O-1	a						
O-2	b						
O-3	c						
O-4	d						
S-1	a						
S-2	b						
S-3	c						
S-4	d						

TABLE 17.2	Sample Data Sheet for Pigment Analysis by Spectrophotometry.

Stream: Date:

Location: Investigators:

Open/Shaded reach	Rep. #	Preacidification		Postacidification		Chl a (μg/cm^2)	Pheophytin (μg/cm^2)
		750 nm	664 nm	750 nm	665 nm		
O-1	a						
O-2	b						
O-3	c						
O-4	d						
S-1	a						
S-2	b						
S-3	c						
S-4	d						

where:

$E_{664b} = [\{$Absorbance of sample at 664 nm − Absorbance of blank at 664 nm$\}$ − $\{$Absorbance of sample at 750 nm − Absorbance of blank at 750 nm$\}]$ before acidification;

$E_{665a} = [\{$Absorbance of sample at 665 nm − Absorbance of blank at 665 nm$\}$ − $\{$Absorbance of sample at 750 nm − Absorbance of blank at 750 nm$\}]$ after acidification;

$V_{ext} =$ Volume of 90% acetone used in the extraction (mL);

L = length of path light through cuvette (cm);

26.7 = absorbance correction (derived from absorbance coefficient for chlorophyll *a* at 664 nm [11.0] × correction for acidification [2.43]);

1.7 = maximum ratio of $E_{664b} : E_{665a}$ in the absence of pheopigments

Autotrophic Index[6]

1. Calculate the Autotrophic Index (AI), which is the ratio of periphyton biomass to chlorophyll *a*

$$AI = AFDM\,(mg/cm^2)/Chlorophyll\ a\,(mg/cm^2) \qquad (17.6)$$

Statistical Comparisons Using *t*-tests (see Zar 1999 or Chapter 35)

1. Compare algal biomass and pigments in open versus shaded reaches.
2. Compare algal pigments on natural versus artificial substrata.

Specific Pigments by HPLC (see Table 17.3)

1. For each standard solution (*β*-carotene, chlorophyll *a*), calculate the slope of the relationship between μg of pigment injected and peak area obtained from the computer integrator. Use this information to produce a calibration curve of the form:

$$peak\ area = intercept + (slope \times mass\ injected) \qquad (17.7)$$

Pigment concentration in the standard (μg/mL) should be provided from the manufacturer (e.g., Sigma). If the concentration is not stated, the concentration X (in μg of pigment in Y mL of solution) can be calculated as:

$$X = (Abs_{max} \times Y) \times (\alpha \times 100)^{-1} \times 10^6 \qquad (17.8)$$

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[6] Remember to convert chlorophyll *a* from μg/cm^2 to mg/cm^2 (divide by 1000) before calculating this index.

TABLE 17.3 Sample Data Sheet for Pigment Analysis by HPLC. Pigments are listed in approximate order of appearance in HPLC chromatogram. Note that this list is not exhaustive, not all compounds will appear in every periphyton sample, and that the order of pigments appearing in a chromatogram may not be consistent among samples. See Millie *et al.* (1993) for more information on taxonomic associations.

Stream:			Date:		
Location:			Investigators:		
Reach Type (O/S):			Replicate #:		
Major pigment	Absorbance maximum (nm)[1]	Expected retention time (min)[2]	Position relative to Sudan[3]	Observed retention time (min)	Peak area	Taxonomic association
Scytonemin	362	2.3	0.31			Cyanobacteria
Chl *c1*	440	2.8	0.38			Bacillariophyceae and others
Chl *c2*	444	3.5	0.47			
Pheophorbide *a*	410	4.7	0.62			Chl *a* degraded (by grazers)
Fucoxanthin	448	4.8	0.64			Bacillariophyceae
Diadinoxanthin	440	7.2	0.96			Bacillariophyceae
Sudan	498	7.5	1.00			standard
Myxoxanthophyll	471	7.8	1.04			Cyanobacteria
Alloxanthin	452	8.6	1.15			Cryptophyta
Diatoxanthin	438	9.1	1.21			Bacillariophyceae
Lutein[4]	445	9.5	1.27			Cyanobacteria + Chlorophyta
Chl *b*	462	13.5	1.80			Chlorophyta
Chl *a*	432	15.3	2.04			All groups
Echinenone	458	16.0	2.13			Cyanobacteria
Pheophytin *b*	436	18.2	2.43			Chlorophyta (degraded)
Pheophytin *a*	410	21.3	2.84			All groups (degraded)
β-carotene	448	22.2	2.96			All groups

Notes:

1. All absorbance maxima are reported for the solvents used by Leavitt and Hodgson (2001) (i.e., mixture of Solvent A and B) at the time the pigment passes through the detector. If you have a photodiode array detector on your HPLC, you can use these maxima to tentatively identify the carotenoid or chlorophyll. Relative chromatographic position is calculated as: time of pigment/time of Sudan II.

2. Retention times can vary substantially, depending on the type of HPLC column used, pumping pressure, solvent composition, and other features. These times should be used only as a rough indication of the expected position of a pigment.

3. Chromatographic position relative to an internal standard, such as Sudan II dye, can be a more reliable measure of pigment identity than the time of peak. Try calculating your own retention times to see if you can determine pigment identity.

4. Lutein (chlorophytes) and zeaxanthin (cyanobacteria) may not be separated on this HPLC system and are presented together.
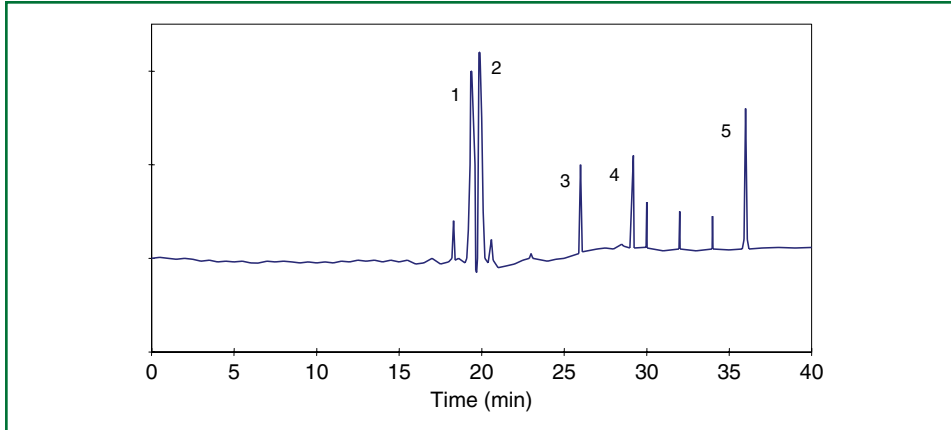
FIGURE 17.3 Representative chromatogram of benthic periphytic pigments, showing relative absorbance of the following pigments: (1) lutein, (2) zeaxanthin, (3) chlorophyll *b*, (4) chlorophyll *a*, and (5) *β*-carotene.

where Abs_{max} = maximum absorbance (at 454 nm for *β*-carotene, 662.7 nm for Chl), α = specific extinction coefficient (*β*-carotene = 2500, chlorophyll *a* = 88.15, both in acetone). For this calculation, measure maximum absorbance of the standard solution in a spectrophotometer using a glass cuvette with a 1-cm pathlength.

2. For each periphyton sample, record the peak area (Figure 17.3) for all pigments listed in Table 17.3. If pigments are absent, record an area of zero.

3. Calculate the mass of each pigment in each sample using the calibration regressions determined above. Assume all carotenoids and scytonemin have properties similar to those of *β*-carotene (i.e., scytonemin, fucoxanthin, myxoxanthophyll, lutein, zeaxanthin, canthaxanthin, echinenone, *β*-carotene). Assume chlorophylls and their derivatives (i.e., scytonemin, chlorophyll *c*, chlorophyll *b*, chlorophyll *a*, pheophytin *b*, pheophytin *a*) have properties similar to those of chlorophyll *a*. These assumptions will introduce small errors into your determination of pigment mass because each pigment has its own, unique absorbance characteristics. However, in most cases, these differences are much smaller than differences in algal biomass among sites, and will not substantially alter the outcome of your analyses.

4. Calculate the total pigment mass (µg) on your substrate as follows:

$$\text{Total } \mu g \text{ pigment} = \mu g \text{ pigment injected}$$
$$\times [\text{total } \mu L \text{ extract (i.e., } 400 \,\mu L)/\mu L \text{ injected}] \qquad (17.9)$$

5. Express pigment mass per cm^2 of substrate.

IV. QUESTIONS

1. If different biomass levels were found between open and shaded reaches, what environmental factors besides irradiance may have accounted for this difference?

2. Were both AFDM and chlorophyll *a* greater in one type of reach or another (i.e., did they show a similar pattern in open vs. shaded reaches)? If not, what might have accounted for the variation?
3. What kind of graphical relationship is found between periphyton biomass and light? Is it positive or negative? Is it linear, logarithmic, hyperbolic? Why?
4. If you were able to conduct this study over an entire year, how might you expect algal AFDM or chlorophyll *a* to change in your stream over the four seasons? What about in a desert stream or a deciduous woodland stream?
5. Some streams with very low periphyton abundance support very large populations of grazing macroinvertebrates. How is this possible?
6. If comparisons of substratum type (i.e., natural vs. artificial) were made, which type gave the most consistent (i.e., lowest variability) pigment results?
7. What are the strengths and weaknesses of analyzing pigments with HPLC compared to a spectrophotometric approach? If you did both, were levels of chlorophyll *a* determined from spectrophotometry and HPLC similar?

V. MATERIALS AND SUPPLIES

Field Materials

> Plastic containers (one per site; large enough to accommodate 10 tiles, labeled)
> Unglazed clay tiles (e.g., 100 tiles measuring 5×5 or 10×10 cm)
> Materials and equipment necessary to measure irradiance (see Chapter 5), dissolved nutrients (Chapters 9–11), and grazer density (Chapters 20 and 23) if the advanced method is conducted

Laboratory Materials

AFDM

> Aluminum foil
> Aluminum weigh boats or porcelain crucibles
> Coarse-bristled toothbrushes
> Tongs (for handling hot crucibles)

Spectrophotometry

> 0.1N Hydrochloric acid
> 90% buffered acetone (90 parts acetone and 10 parts saturated magnesium carbonate solution)
> Forceps
> Kimwipes
> Pipettes (5 or 10-mL and Pasteur)
> Saturated magnesium carbonate solution (1.0 g finely powdered $MgCO_3$ added to 100 mL distilled water)
> Black film canisters or scintillation vials (100 20-mL volume, labeled)
> Squirt bottles
> Whatman GF/F filters, or equivalent (100)

HPLC

> 90% buffered acetone, as above
> 100% acetone
> 5-mL plastic syringe and locking filter (0.2-μm pore size, chemically-resistant)
> Pasteur pipettes, rubber bulb
> 10-mL glass sample vials and caps
> N_2 gas tank, gas regulator and plastic hose
> HPLC sample vials and caps (4-mL volume, labeled)
> Flat-tip 100-μL Hamilton syringe suitable for HPLC injector (if no autosampler)
> Ion-pairing solution (7.77 g tetrabutyl ammonium acetate and 0.75 g ammonium acetate in 100 mL distilled water)
> Injection solution with internal standard (e.g., Cu-Meso-IX-DME; Steinman *et al.* 1998; Sudan II dye; Leavitt and Hodgson 2001). Injection solvent is 70% acetone: 25% ion-pairing reagent: 5% methanol (by volume) containing 3.2 mg Sudan II/L
> Mobile phase solvents, including solvent A (10% ion-pairing solution in methanol) and solvent B (27% acetone in methanol)
> Pigment standards dissolved in injection solvent, including β-carotene and Chl *a* (Sigma Chemical Co.)

Laboratory Equipment

AFDM

> Analytical balance (sensitivity of 0.1 mg)
> Desiccator
> Drying oven
> Filtration apparatus with vacuum pump
> Muffle furnace

Spectrophotometry

> Filtration apparatus with vacuum pump and solvent-resistant filter assembly
> Cuvettes (1-cm pathlength)
> Spectrophotometer (narrow band width: 0.5 to 2.0 nm)

HPLC (minimum requirements)

> HPLC autosampler or injector
> HPLC solvent pumps (2)
> In-line spectrophotometer or photo-diode array detector
> Digital integrator
> Computer controller and associated software
> HPLC column (10 cm; C-18 reversed phase; 5-μm particle size)
> Centrifuge
> Eppendorf pipette (1–100 μL capacity)
> Cuvettes (1-cm path length)
> Spectrophotometer (narrow band width: 0.5 to 2.0 nm)
> Filtration apparatus with vacuum pump and solvent-resistant filter assembly

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Macrophytes and Bryophytes

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I. INTRODUCTION

Aquatic macrophytes and bryophytes have structures that are usually more complex, interdependent, and physically substantial than benthic algae (Chapter 16). In this chapter, macrophytes are defined as aquatic vascular plants in that they have internal structures (*xylem* and *tracheids*) to transport water and nutrients through the organism and typically have true roots. Bryophytes include mosses and liverworts, which do not have xylem and lack true roots as in higher plants but do have differentiated cells in each individual. Thus defined, macrophytes and bryophytes are distinct from algae, which lack a vascular system and true roots and have individual cells that are largely independent and undifferentiated, even when in a colonial form.

Where macrophytes and bryophytes are abundant, they can profoundly influence the structure and function of stream ecosystems (The Stream Bryophyte Group 1999, White and Hendricks 2000). For example, these organisms influence the physical environment by modifying water current and sediment conditions. They can also alter the abundance and community structure of stream fauna and have the potential to compete effectively for resources such as space, nutrients, and light. Relatively little research has been done on the ways in which bryophytes affect fundamental ecosystem processes such as production, decomposition, and nutrient regeneration (The Stream Bryophyte Group 1999). In comparison, the literature on stream macrophytes is more diverse and advanced (e.g., Prahl

et al. 1991, White and Hendricks 2000, Kaenel *et al.* 2000, Riis *et al.* 2001, Dodds and Biggs 2002, Riis and Biggs 2003).

A. Macrophytes: An Overview

Classification of Stream Macrophytes. Macrophytes are literally "large plants," which some researchers (e.g., White and Hendricks 2000) interpret to include large filamentous algae (e.g., *Batrachospermum* spp. and the Characeae), bryophytes, and liverworts, as well as higher plants. For the purposes of this chapter we will define macrophytes more narrowly as vascular plants in which specialized cells (*tracheids*) transport water and minerals from true roots. Bryophytes and liverworts will be treated separately in this chapter. Benthic algae are considered in Chapter 16.

The primary generation phase in macrophytes is the *sporophyte*, which has two sets of chromosomes per cell (i.e., it is *diploid*). Freshwater macrophytes thus defined are often classified based on life-form: emergent plants, floating-leaved plants, submerged plants, and free-floating plants (Table 18.1; Sculthorpe 1967). A classification based on life-forms is useful in large rivers and rivers with strong floodplain connections, where all of the life-forms are often present. However, this might not be the best classification system for small, wadeable streams where continuous and often strong currents and a lack of slow-flowing environments such as meanders and backwaters tend to limit

TABLE 18.1	Traditional Classification of Macrophytes and the Classification Recommended in This Chapter for Stream Macrophytes.
Traditional Classification of Aquatic Plants	**Characteristics**
Emergent plants	Plants normally erect and standing above the water surface, but some species tolerate submergence; all produce aerial reproductive organs
Floating-leaved plants	Plants permanently submerged and produce floating leaves that differ in morphology from submerged leaves in still and slow-flowing water; produce floating or aerial reproductive organs
Submerged plants	Plants permanently submerged; produce floating, aerial, or submerged reproductive organs
Free-floating plants	Plants not attached to the substrate; produce floating, aerial, or very rarely submerged reproductive organs
Classification of Stream Macrophytes	**Characteristics**
Obligate submerged plants	Similar to submerged plants above but also includes the floating-leaved plants
Amphibious plants	Part of the emergent plants above; able to live on land as well as emerged and submerged; some develop water forms
Terrestrial plants	Mainly present on land; occasionally in streams; do not live permanently submerged; never develop water forms

free-floating and floating-leaved species. Moreover, the emergent plants present in small wadeable streams include species that can tolerate a range of conditions from permanent to only occasional submergence. In these cases it is appropriate to further differentiate the emergent life-form into more groups.

One useful alternative is to classify stream macrophytes on the basis of the primary habitat in which particular species are found. At the stream edges and in riparian habitats one often finds plants that are able to live on dry or moist land but can tolerate at least some (often prolonged) inundation. As one moves to deeper habitats that are more permanently submerged, the form and types of species usually shifts. It is therefore useful to classify stream macrophytes into obligate submerged plants, amphibious plants, and terrestrial plants based on their primary habitat (Sand-Jensen *et al.* 1992, Riis *et al.* 2001). Obligate submerged plants live permanently submerged in the streams and are only rarely present on the banks (e.g., common examples include *Potamogeton* sp. and *Myriophyllum* sp.; Table 18.1). Amphibious plants are able to live on land in a fully emergent state but can also live fully submerged. Some species develop water forms, which are morphologically distinct from the land forms (e.g., *Veronica anagallis-aquatica*, *Sparganium emersum*). Terrestrial plants live mainly on land but can occasionally be observed within streams. However, terrestrial plants never form permanently submerged populations and do not develop morphologically distinct water forms (e.g., *Epilobium hirsutum*).

Distribution and Abundance of Stream Macrophytes. The macrophytes that are present at a particular site in a stream are the result of interactions among a variety of factors, including the physiological demands of the plant, the ability of the plant to tolerate the local environmental conditions, the dispersal history of the plant, and interactions with other plant species. Thus, a plant species must be able to establish, survive, grow, and reproduce under the prevailing environmental conditions to be sustainable in a stream site.

The physical environment — in particular, current velocity and flow regime — has a strong influence on macrophyte establishment and success. For macrophytes to be present at a site, they have to establish from seed, vegetative propagules, or by expansion from neighboring populations. If a macrophyte population establishes from seed or vegetative propagules, the water velocity must allow these units to settle at the site. This typically requires low current velocities ($<0.1\,\mathrm{m\ sec^{-1}}$ depending on the buoyancy of the seed or propagule) or fortuitous circumstances (i.e., an obstacle that entraps the seed or propagule). If macrophytes colonize a site by expanding from a neighboring population, they will succeed only if the water velocity does not exceed thresholds that would cause substrate erosion or that would cause macrophyte stems to break due to drag forces. The threshold for substrate erosion depends on the particle size and stream bed armoring. Chambers *et al.* (1991) found that vegetation was absent from Canadian rivers they examined when the mean water velocity was greater than $1\,\mathrm{m\ sec^{-1}}$ and Henriques (1987) found no vegetation in 22 New Zealand streams where mean velocity exceeded $0.9\,\mathrm{m\ sec^{-1}}$.

The frequency of floods also affects macrophyte presence, abundance, and species diversity in streams. In a study of 17 New Zealand streams, the abundance and diversity of macrophytes decreased as flood frequency increased and vegetation was absent in streams with more than 13 high-flow disturbances per year (flows 7 times greater than the mean annual flow, Riis and Biggs 2003). These results suggest that prolonged periods of hydrological stability are required for macrophyte propagules (e.g., seed, stem fragments,

or dispersal organs, *turions* and *stolons*) to arrive and develop into substantial cover. Riis *et al.* (2004) examined macrophyte colonization in hydrologically stable artificial streams with no *in situ* seed bank or adjacent macrophyte beds, but with an upstream source of vegetative propagules. They found that 10–20 weeks were required for macrophytes to establish >1% cover. Thus, a long interflood period is required to allow vegetation to develop to the maximum possible biomass. Even a small number of floods in a stream within a year will decrease macrophyte abundance compared with streams without flood disturbances.

The effect of light on stream macrophyte distribution and abundance can be substantial (Kern-Hansen *et al.* 1980, Carr *et al.* 1997, Haury and Aidara 1999, White and Hendricks 2000). Because of shading, only a few macrophytes are able to live in forested streams with substantial canopy cover. In deep or turbid streams, light can limit macrophyte growth because insufficient light reaches the stream bottom. Self-shading often occurs in dense macrophyte stands, which limits growth and further biomass accumulation. Seasonal changes in macrophyte biomass in temperate streams (Champion and Tanner 2000, Riis *et al.* 2003) are also associated with a combination of changes in light and temperature between summer and winter.

In addition to light, all plants require nutrients and inorganic carbon for growth. Lowland streams often receive water enriched with nutrients and carbon dioxide from the catchment. These resources are recycled or replenished relatively quickly and so nutrients and carbon are usually not major factors that control macrophyte distribution and biomass in streams. Moreover, many macrophytes can use bicarbonate as a carbon source, which makes them even less susceptible to carbon limitation. It is also important to note that macrophytes can assimilate nutrients and carbon through both leaves and roots. Many studies have shown that sediment interstitial water is a significant source of nitrogen and phosphorus (e.g., Bristow and Whitcombe 1971, Barko and Smart 1981), while others have shown that in nutrient-rich systems, stream macrophytes are able to satisfy their nutrient demand by leaf uptake alone (Madsen and Cedergreen 2002). Most likely, the relative importance of roots and leaves in nutrient uptake depends on the nutrient availability in the sediment and the water (Carignan 1982), rather than species-specific physiology. Thus, plants growing in nutrient-poor streams are able to meet their nutrient demand by assimilation through roots. Current velocity may also strongly influence nutrient uptake through macrophyte leaves. Under stagnant conditions, nutrients in water at the boundary of the leaf surface can become depleted and the plant may shift to root assimilation. Thus, vigorous water flow in streams may favor nutrient uptake through the leaves.

In addition to abiotic factors, biotic factors such as competition and herbivory can affect the distribution and abundance of stream macrophytes. Competition among macrophyte individuals or populations is likely to occur in nondisturbed sites with high macrophyte abundance. In sites with regular disturbance, the vegetation might never reach a density where space is a limiting resource. Some insect larvae can feed on macrophytes (Lodge 1991, Newman 1991, Jacobsen and Sand-Jensen 1992, 1995). However, it does not seem that herbivory is a major factor that controls macrophyte distribution and abundance in most streams.

Ecological Role of Stream Macrophytes. In stream ecosystems, macrophytes are most often found in soft-bottomed habitats. Where macrophytes are present they have important influences on stream ecology by affecting bed substrate composition and modifying local flow conditions (Figure 18.1). Water velocity is reduced in macrophyte beds and
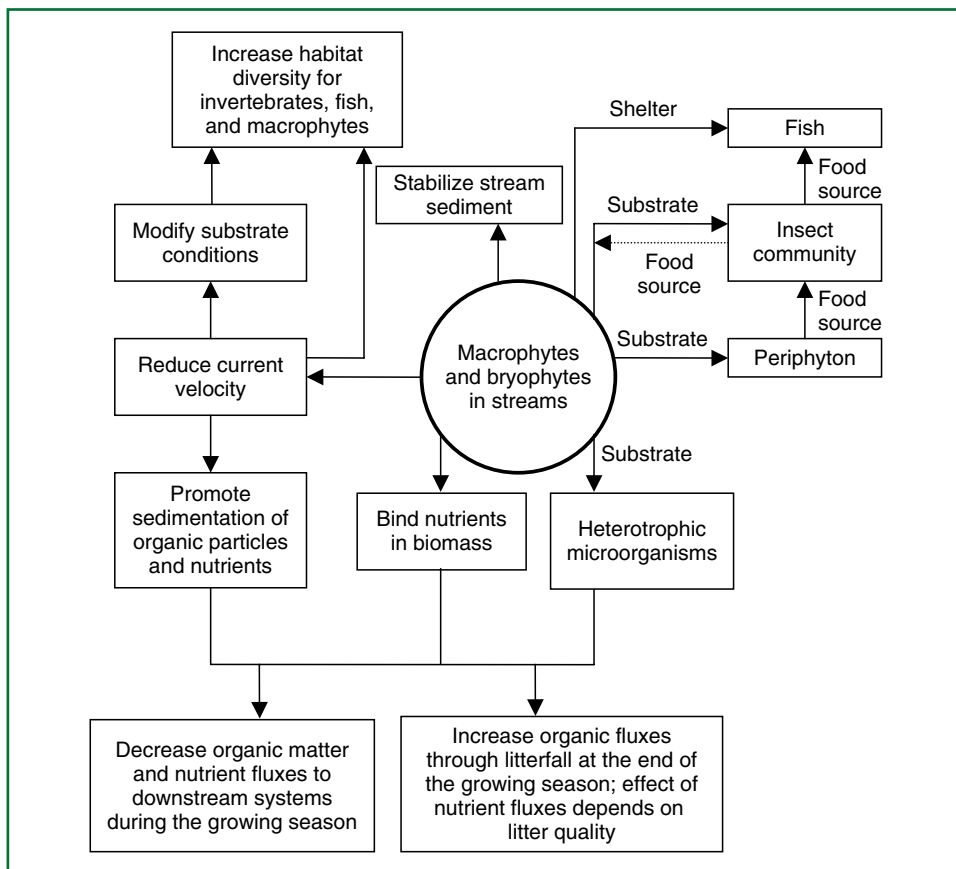
FIGURE 18.1 Conceptual model describing the main ecological influences of macrophytes and bryophytes in stream ecosystems. The effects of macrophytes and bryophytes in stream ecosystems are similar in many ways. One of the most important differences may be their effects on the nutrient quality of organic matter produced through litterfall. Compared to macrophytes, very little is known about the ways in which bryophyte litter decomposes. Because litterfall is a critical link in the recycling pathway and a major resource for different components of the stream food web, this potential difference between macrophytes and bryophytes is particularly interesting. See The Stream Bryophyte Group (1999) for further discussion of this topic.

fine particulate matter settles to the bottom (Sand-Jensen and Mebus 1996, Sand-Jensen 1998, Clarke 2002) where macrophyte roots help to stabilize the bed. Organic matter and associated nutrients tend to be recycled rapidly by the rich invertebrate and microbial communities in macrophyte beds (Chambers and Prepas 1994, Clarke 2002). Macroinvertebrates feed on the abundant microorganisms and periphyton, and perhaps directly on macrophyte tissues; though macroinvertebrate diversity may be lower in some macrophyte environments. As a consequence, fish tend to be more abundant in streams where macrophytes are also abundant because there is more food and better cover (i.e., hiding places). Overall, the presence of macrophytes creates more diverse stream habitats which benefit a wide range of other stream organisms.

B. Bryophytes: An Overview

Classification and Life Cycle of Stream Bryophytes. Bryophytes include liverworts (Marchantiophyta = Hepatophyta, formerly Hepaticae), hornworts (Anthocerotophyta, formerly Anthocerotae), and mosses (Bryophyta, formerly Musci). Bryophytes have only one set of chromosomes, lack lignin for support, do not have tracheids, and lack true roots. The liverworts differ on their upper and lower surface, whereas mosses are similar all the way around the stem and may grow upright or horizontally. Most (but not all) stream mosses have a horizontal growth form. Identification of bryophytes to the level of species can be technically involved, depending on the species present. Nevertheless, Appendix 18.1 is a simple key that can be used to identify important and common genera of aquatic bryophytes based on morphological characteristics that are reasonably easy to see.

Interestingly, the bryophyte plant that one sees in a stream has only one set of chromosomes (i.e., it is *haploid*) and is actually either a "male" plant or a "female" plant (as in *Fontinalis*), or both on the same plant (as in *Schistidium*). More highly evolved plants like macrophytes have two sets of chromosomes (i.e., they are *diploid*). Bryophytes are unusual in having "generations" that alternate between a relatively long haploid generation and a relatively short and less prominent diploid generation. At maturity, the female part of the bryophyte population produces sexual organs called *archegonia* that produce eggs, and the male part of the population produces sexual organs called *antheridia* that produce sperm. The egg and sperm are both gametes and so this stage is part of the haploid gametophyte generation of the bryophyte life cycle. Sperm cells from the antheridia fertilize eggs in the archegonia to produce the diploid *sporophyte*, which remains dependent upon the gametophyte, another feature that is unusual in bryophytes. The mature sporophyte is composed of a stalk (*seta*) and a spore case (*capsule*). These are often covered by a cap (*calyptra*), which keeps the capsule closed until the spores are ready for dispersal. Physical characteristics of the sporophyte (some of which can be quite subtle) are key characteristics used to identify bryophyte species. However, these capsules are seldom present in aquatic species.

Although sexual reproduction is clearly important in bryophytes, they can also reproduce vegetatively. That is, pieces of the "plant" may break off when disturbed (e.g., by floods) and the pieces can float downstream to establish in a new location. Vegetative reproduction may be particularly important for stream bryophytes and is a major means by which some species disperse.

Liverworts have two general forms: *thalloid* or *leafy*. Thalloid liverworts have no true stems or leaves and cells form an apparently disorganized mass (the *thallus*), although in some species there may be some internal differentiation (see Appendix 18.1). Antheridia and archegonia may be imbedded in the thallus or raised on a stalk made of thallus tissue. Leafy liverworts, in contrast, have a definite stem with leaves that are one cell thick (see Appendix 18.1). The leaves are arranged in two rows, giving many taxa a flattened appearance that may trend up toward the tip (*incubous*) or down from the tip (*succubous*). A third row of usually smaller leaves may be present on the ventral surface. Characteristic pockets, folds, lobes, or other forms of leaf modifications also may be present on the lower surface of the paired leaves and are important characteristics used to identify some species. Both liverwort forms (with a few exceptions) have a distinct "up-down" (i.e., *dorso-ventral*) construction, unicellular *rhizoids* (threadlike structures that aid in attachment and absorption), and capsules with *elaters* (threadlike structures that are generally thought to aid in dispersal) among their spores.

Mosses differ from liverworts in lacking the dorso-ventral orientation, although many species lie prostrate across the substrate and produce rhizoids only on the lower surface. True mosses always have differentiated leaves and stems (see Appendix 18.1). Antheridia, archegonia, and capsules are borne at the apex (*acrocarpous*) in the upright taxa or on short lateral branches (*pleurocarpous*) in the more prostrate taxa. No elaters are present in the capsules, but most capsules have teeth (*peristomes*) at their opening to help regulate dispersal. Their leaves are seldom two-ranked, with a spiral arrangement being more common. Moss species are typically identified on the basis of the shape, color, and texture of leaves and leaf cells and (when present) the appearance, shape, and structure of the sporophyte. The presence of rhizoids, which are multicellular, seems to be habitat-dependent. Terrestrial and stream mosses tend to have rhizoids, but mosses in quiet water often fail to produce them, although Lodge (1959) showed that some of these taxa will produce rhizoids when the plants are out of the water. Many moss taxa have rudimentary internal conducting cells (*hydroids* and *leptoids*), but these cells are generally absent among the aquatic species.

Excellent summaries of the general structure, life cycle, and classification of bryophytes may be found in the North American field guides by Conard and Redfearn (1979) and Vitt *et al.* (1988). These field guides are widely available and serve as useful entries to more technical taxonomic keys, which are listed as general references in these two publications. The authoritative taxonomy of North American bryophytes is the two-volume set by Crum and Anderson (1981). Although oriented to the Eastern United States, it is useful in other locations in North American and beyond. For fieldwork in North America and Europe, the short key provided in Appendix 18.1 will help identify some of the bryophyte genera most often encountered in stream environments.

Distribution of Stream Bryophytes. Stream bryophytes do not have true roots and are *poikilohydric* and thus they rely entirely on absorption of water through their leaves. As for terrestrial bryophytes, aquatic bryophytes use a C_3 photosynthetic pathway (Bode 1940, Bolhar-Nordenkampf 1970, Rundel *et al.* 1979, Bain and Proctor 1980, Rudolph 1990). They cannot use bicarbonates as a direct carbon source, unlike many algae and higher aquatic plants, including stream macrophytes (Steeman-Nielsen 1942, 1947, Steeman-Nielsen and Kristiansen 1949, Bain and Proctor 1980, Allen and Spence 1981, Maberly 1985, Raven 1991, Madsen *et al.* 1993). Thus, bryophytes are often restricted to acidic stream waters (Frahm 1992) where dissolved CO_2 is abundant. They are known to exist in waters with pH < 3 (Hargreaves *et al.* 1975) and can be the dominant plants in acidic environments (Sand-Jensen and Rasmussen 1978). However, other species like *Fissidens grandifrons* and *Cratoneuron filicinum* are calciphiles and seem to prefer nonacidic environments.

In contrast to stream macrophytes, stream bryophytes tend to live in faster and shallower water, in part due to their need for constant replenishment of carbon dioxide and nutrients directly from the water. They also prefer hard-bottom substrates (e.g., cobble, boulders, and ledge) because they attach to the surface of substrates via rhizoids and not by roots that penetrate soft sediments. As a consequence, bryophytes are often abundant in riffles, in the splash zone of emergent rocks and stream banks, and in turbulent cascades and waterfalls.

Bryophytes are primary producers (*autotrophs*) like benthic algae and macrophytes and so they require light to grow. Light influences bryophyte growth and distribution in much the same way as it does macrophytes. However, many bryophytes have an ability to continue to photosynthesize at relatively low light levels, although most of what we know about this characteristic of bryophytes comes from lake rather than stream literature

(e.g., Middelboe and Markager 1997, Riis and Sand-Jensen 1997, Sand-Jensen *et al.* 1999, Schwarz and Markager 1999). Thus, unlike macrophytes, bryophytes may be abundant even in forested headwater streams with dense leaf canopies (Glime 1970, 1984).

Individual bryophyte species can be widely distributed around the world. This seems to be especially true of the more limited set of bryophytes species that are most often found in aquatic habitats. Similar, or even the same, species can be found in North America, Europe, and Austral-Asia.

Ecology of Stream Bryophytes. The literature on the role of bryophytes in stream ecosystems is especially sparse, which suggests that this is an area that is ripe for future research. Because of their ability to bind ions internally and externally, bryophytes have been used to clean up streams and to monitor for intermittent spills (both intentional and accidental) that cannot be detected by chemical means because of their unpredictable nature (Glime and Keen 1984, Cenci 2000). Since stream bryophytes are perennial and resistant to decay, they can remove nutrients and heavy metals and sequester them for a very long time (Ozimek 1988, The Stream Bryophyte Group 1999, Samecka-Cymerman *et al.* 2002). They also have high contents of secondary compounds that discourage both herbivory and microbial breakdown, which prevents substances that have been sequestered by bryophytes from reentering the water column or food chain (Davidson *et al.* 1989, Liao & Glime 1996). These characteristics can have major impacts on the nature of a stream ecosystem.

Many of the statements about the ecology of macrophytes in streams are equally true of stream bryophytes (Figure 18.1). Both provide an extensive surface for colonization by algae and bacteria. Both can affect the flow velocity in streams and, thus can affect sediment dynamics. Both provide habitat for invertebrates and, so, can be a rich source of food for fish (though it is less clear that this is always the case when bryophytes are abundant). However, much less is known about the production, decomposition, and nutrient turnover in bryophytes compared to macrophytes. The scant literature that does exist suggests that the trophic structure and ecological functions of streams that are dominated by bryophytes should be substantially different from those in which bryophytes are not abundant. The review by the Stream Bryophyte Group (1999) discusses these differences at length and provides a convenient entry point to this literature.

II. GENERAL DESIGN

A. Site Selection

The general experimental design and selection of sites for fieldwork on macrophytes and bryophytes will depend on the purpose of the study. Specialized studies of nutrient uptake, photosynthesis, and decomposition often require specialized equipment and analyses that are beyond the scope of this chapter. Appropriate technical literature should be consulted for such studies. However, these specialized studies typically rely on general assessments of macrophyte and bryophyte community characteristics that should be of broad use and interest. These general ecological studies of macrophytes and bryophytes can be included in one of the following study approaches:

1. *Effect analysis* — testing how one or more abiotic or biotic factors affect macrophyte or bryophyte community structure and dynamics (e.g., testing the effect of nutrient enrichment on macrophyte or bryophyte growth)

2. *Temporal analysis* — studying the same community over time (e.g., monitoring short-term seasonal changes or long-term environmental changes).
3. *Pattern analysis* — studying the vegetation patterns in a large number of stream sites without an *a priori* expectation as to what factors as control distribution and abundance of the macrophytes or bryophytes.

In each of these study approaches, replication of experimental sites should be included. For example, when the study calls for an effect analysis (approach 1), the sites could be stratified into groups — one group with stream replicates but without the effect and one group with stream replicates including the effect. Replication of study sites within a group is critical for an effect analysis, to distinguish the target effect from other (often unknown) effects that might confound the experimental conclusions. Alternatively, the effect can be studied using a correlation analysis by investigating a range of stream sites affected to various degrees by the target effect.

In addition, consideration should be given to reference or control sites that can be used to assess how the "experimental" stream differs from the "normal" reference condition. For example, to test how a change in an environmental factor will change the macrophyte community over time (a temporal analysis, approach 2), it is necessary to select sites where the environmental factor is known to be changing, as well as reference sites (preferably in the same stream system or at least same region) where the environmental factor is not changing.

When choosing study sites, try to ensure that there are no obvious ancillary factors that could overwhelm the key factor that is the focus of the experiment. For example, shade from riparian vegetation can have a profound influence on the vegetation in a stream. If shading is not the focus of your study, ensure that all study sites are shaded to a similar degree.

In this chapter, the focus is on macrophyte and bryophyte communities within stream reaches. For this purpose, selecting a "representative" reach is important. The particular characteristics that are representative of streams in one region or location may differ from those in other regions or locations. Refer to Chapters 1 and 2 (on landscapes, basins, streams, and reaches) for ideas and concepts to consider. For most low-order, wadeable streams, a reach of 100 to 200 m will probably encompass most of the reach characteristics of interest. Assess the general shape and form of your stream and select a reach (or reaches) that include most of the common features (e.g., pools, runs, riffles, steps, cascades). Ideally, each reach should include several of each of these common features. Interesting experiments can be devised by comparing representative reaches at different locations (stream orders) within a single network or among several streams that have different land use or land cover characteristics.

Prior to going to the field, try to consult topographic, soil, and surficial geology maps, satellite imagery, and/or black and white orthophotographs to identify likely sampling sites. These resources can often be found at agencies that are charged with managing natural resources and in university libraries. Increasingly, these resources can be found on the internet.

B. Field Sampling

Cover or *abundance* is one way to address how commonly macrophytes and bryophytes (or individual species of either group) occur in streams. Most of the wide variety of techniques commonly used for this purpose in terrestrial vegetation analyses can be used

in streams with only minor modifications (e.g., Kent and Coker 1995). Approaches that rely on visual cover estimation (e.g., plot assessments) or quantitative cover measurement (e.g., point transects) are useful, quick, and inexpensive.

Visual cover estimations derive from an approach developed by the phytosociologist Josias Braun-Blanquet (1932). The core of this approach is a classification system based on the estimated (visual) abundance of a species in a defined area (Figure 18.2 and Table 18.2). Other classifications with other intervals have been proposed, but the approach is similar. Due to the subjective nature of this approach it is best that one person does all of the visual plot assessments. An alternative is to have two or more observers simultaneously estimate the visual cover of a species and then "negotiate" an agreed value to record. When multiple people are involved in the study, but do their
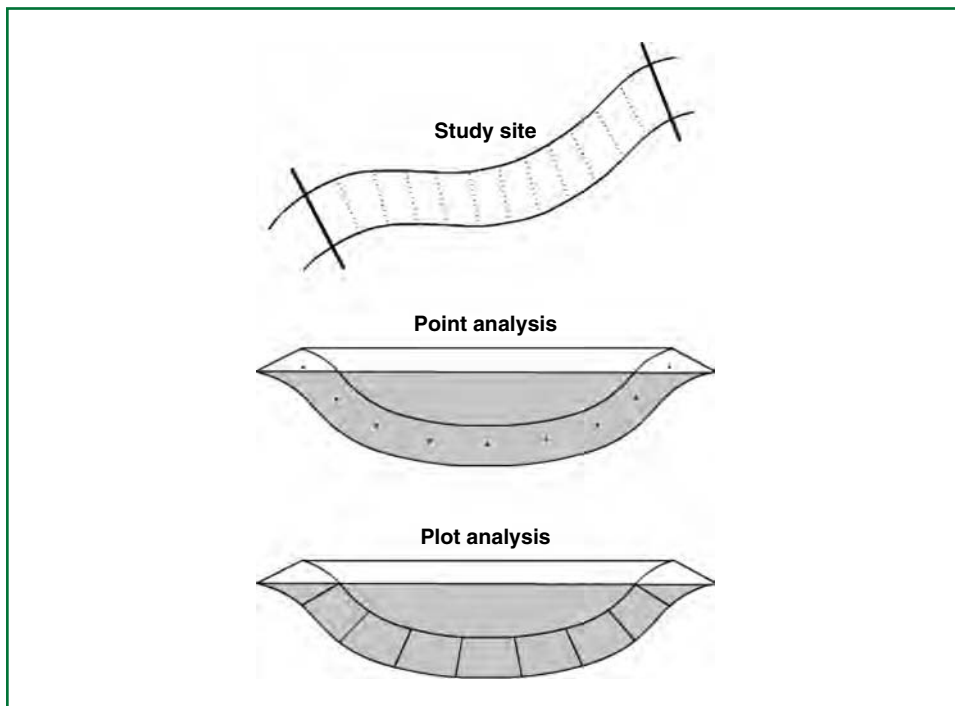
FIGURE 18.2 Sketch of stream site (bold lines) with marked transects (dotted lines) for use in macrophyte/bryophyte studies. Enlargements are for point and plot analyses in transects.

TABLE 18.2	Braun-Blanquet Cover Scale.
Cover Scale Classes	**Percentage Cover**
1	<5%
2	5%–25%
3	25%–50%
4	50%–75%
5	75%–100%

assessments separately, it is essential to intercalibrate the individual assessments for some (5–10%) of the observed plots.

An alternative approach is to estimate cover and abundance from "point transects," which is a modification of the point frame method used in terrestrial vegetation analysis. In this method, the type of cover observed beneath specific points spaced at regular intervals is recorded (Figure 18.2). The spacing of the intervals depends on the size of the stream, but intervals of 5 to 50 cm are appropriate for streams from a few meters to 20 m wide (or streams too deep to wade). The number of times that a particular cover type (e.g., a macrophyte or bryophyte species) is "hit" is used as an estimate of cover, as described in the following detailed methods. The point transect method can be modified easily to accommodate as many characteristics as you wish. Thus, the same approach can be used simultaneously to estimate cover by species, percentage of flowering versus nonflowering individuals, substrate quality, or any other characteristic that can be identified at a short distance. By simply changing the orientation and sampling interval, this approach can be used for intensive, site-specific characterizations or for extensive, longitudinal characterizations of streams.

A plot-based approach increases the chance that you will include rare species in the assessment, as a larger area is surveyed and large areas have more species than small areas. On the other hand, making plot observations takes more time and so normally, fewer plot observations can be made compared to point measurements.

Biomass can be estimated using quadrats, which are rigid frames that encompass a known area. The shape and size of the quadrat frame does not matter as long as the enclosed area is known and the quadrat is sufficiently weighted so that it does not float in the stream current. The quadrat is deployed randomly (through some rules or by literally tossing it in a general sampling area) and all macrophyte or bryophyte biomass that falls within the quadrat is removed; washed briefly in the stream to remove loosely adhering algae, detritus, and sediment; sorted by species (if desired); and returned to the laboratory for further analysis. Because the clipped biomass will be wet, it is better to use prelabeled plastic bags to temporarily store the samples in the field. Use an indelible marker to label the bags prior to taking the sample. Before placing the wet biomass in the bag, allow it to drain and, if possible, gently wring out excess moisture. Store the samples cold and in the dark (e.g., in a cooler). Never leave fresh samples in plastic bags for more than 24 h before processing them further or they may begin to mold and decompose.

Remember that biomass sampling is destructive. Therefore, if you intend to sample in an area known to have rare or slow-growing species, take care to strictly limit the total mass of sample you remove. Endangered species should never be sampled.

C. Laboratory Processing

Voucher Specimens. Usually there is insufficient time when doing fieldwork to unequivocally identify species in hand. Even experts would have difficulty identifying some species whose distinguishing features can only be identified under a microscope. It is helpful, therefore, to save representative specimens to examine at leisure in the laboratory. These specimens are called "voucher" specimens. While in the field, these specimens can be assigned a temporary name (e.g., "unknown1," "unknown2," etc.) and when the specimens have been reliably identified the confirmed name can be used to fill in the "unknowns."

The techniques used to collect, prepare, and store voucher specimens of macrophyte and bryophyte species differ slightly for practical reasons. However, in both cases, the

objective is to collect specimens that will provide as many clues as possible when you finally have the time to examine them in detail. For macrophytes, it is especially helpful to have flowers and seeds in addition to leaves, stems, and roots. For bryophytes, it is especially helpful to have the sporophyte (seta and capsule). Reliable identifications can be made on the basis of leaf and stem material alone for some species. However, for others (e.g., some species of *Carex* macrophytes or *Bryum* bryophytes) it may only be possible to identify the specimen to the genus level without additional distinguishing features.

Most macrophytes can be collected, prepared, and stored as you would for terrestrial species. A plant press can be used to protect and dry macrophyte specimens until they can be stored more securely in specimen folders. Ordinary office manila folders can be used in lieu of plant specimen folders. The field guide by Conard and Redfearn (1979) has a good description of how to prepare and store bryophyte specimens. Simple envelopes can be constructed from ordinary bond paper. Fold a sheet in thirds as you would to mail a letter and then open the top third. Fold ~3 cm of the left and right edges in toward the center to form a pouch. Insert the specimen and fold the top third down to close and secure the pouch. Field moist samples can be left to air dry or can be oven (70°C) dried directly in these envelopes.

Each specimen should be clearly identified with information that will help you (or someone else) understand the history and characteristics of the specimen. Information you should consider recording on the folder or envelope include:

- The species name (Latin and common names)
- Name of the person who collected the specimen
- Collection number (may be arbitrary or part of a larger system)
- Date of collection
- Name of the person who identified the specimen
- Status of the identification (e.g., provisional or confirmed)
- Habitat of collection (e.g., substrate, flow regime, exposure)
- Location of collection: elevation and coordinates (e.g., latitude, longitude)
- Locality of collection: town, county, state, country
- Any other characteristics or information that might help identify or explain the specimen

Processing for Biomass Analyses. Fresh macrophyte and bryophyte tissues will decompose rapidly and need to be stabilized for further analysis. The most common way to do this is to dry the tissues to a constant weight (70°C). Prelabel a paper bag of a sufficient size to easily accommodate the biomass sample. Remove the field-moist samples from the plastic bag used for the initial collection and transfer them to a small plastic tub partially filled with water. Gently rinse the vegetation to wash away remaining, loosely adhering sediment and organic matter. Change and discard the water as needed. Gently wring or drain the biomass sample to remove excess water and transfer it to the paper bag. The paper bag will wick moisture from the biomass and speed the drying process. Ideally, place the paper bags, open, in a drying oven set to 70°C or, if none is available, in a regular oven set to bake at 160°F (with the door cracked open just a bit). Although some moisture may remain in the tissues even at 70°C, it has been found that at higher temperatures some nitrogen may be volatilized. Thus, if the tissues will be analyzed for total nitrogen it is best not to exceed this temperature. Small quantities (10 to a few hundred grams) of biomass will probably dry adequately in 24 h. However, larger

quantities may take longer to dry and should be checked over a few days to ensure that the weight has stabilized (additional moisture is not being driven off). Use a balance that has a resolution sufficient to weigh the dry biomass to within 2–3% (i.e., for dry samples that end up weighing ~10 g, a balance that can weigh to ±0.1 g is adequate). For bryophytes, a closed balance with a desiccant is helpful because bryophytes gain water from the atmosphere rapidly.

D. Data Reduction and Analysis

Abundance or Cover Data. Cover estimated visually using the Braun-Blanquet or similar methods requires no additional data reduction. If abundance or cover are estimated by the point transect method, the point observations must be converted to percent cover (C%), as follows:

$$C\% = (N_i / N_t) \times 100 \tag{18.1}$$

where N_i is the number of observed points that match the class type i (hits) and N_t is the total number of points observed. These data can then be analyzed as for the visual estimation methods.

Simple Analysis of Abundance Data. If observations have been recorded from replicate plots, the means and standard deviations of the replicates can be calculated. If further statistical comparisons are intended (e.g., ANOVA), it should be noted that these abundance data are percentages and as such are not normally distributed. Standard data transformations (i.e., the arcsine-square root transform, Zar 1999) are commonly used to normalize percent data.

In addition to abundance measures, macrophyte and bryophyte community structure can be described by a variety of simple measures including species richness, species diversity, and species composition.

Species richness is determined by simply counting the species present in the whole study site, each transect, or plot. Simple statistical comparisons can be made among plots, transects, or sites.

Species diversity differs from species richness in that species diversity describes not only species richness but also the relative abundance of the species in a community. A number of different diversity indices exist, but one of the most common is the Shannon-Wiener index (a.k.a. Shannon diversity, H′):

$$H' = -\sum_{i=1}^{S} (p_i \ln p_i) \tag{18.2}$$

where S is the number of species in the sample, and p_i is the proportional (relative) abundance of the *i*th species. The value is typically between 1.5 and 3.5. High values occur when species richness is high and most species are equally abundant in the sample.

The latter is also called 'evenness' because a species sample will appear most diverse when its individuals are evenly distributed among all species. Evenness J is calculated as:

$$J = \frac{H'}{ln\,S} \qquad (18.3)$$

Species composition describes the relative abundance (or just presence or absence) of species in a particular habitat or environment and is often used as an indicator of habitat type or ecosystem health. For example, certain species are known to flourish only under relatively benign or pristine conditions while others are known to tolerate harsh or polluted environments.

Differences in species composition among many sites provide the basis for much more powerful analyses of community structure, including ordination. A discussion of these techniques (which include detrended correspondence analysis, principal components analysis, and canonical correlation analysis) is beyond the scope of this chapter. Those interested in these techniques should consult texts on vegetation analysis (e.g., Kent and Coker 1992).

Simple Analysis of Biomass Data. Cover and biomass estimates can be combined to extend the utility of these data. Cover estimates (by either method) are relatively easy to make. Biomass estimates require greater effort and time. However, in general, biomass is related to cover or abundance; that is, as the abundance of an organism increases so does its biomass. Visual estimates of cover in plots can be recorded before harvesting the biomass from the same plots and then the biomass and cover data can be combined in a simple regression equation (common in most calculators and spreadsheet programs) to provide a predictive equation:

$$Biomass\,(g/m^2) = (slope) \times (C\%) + intercept \qquad (18.4)$$

In this way, more extensive cover data can be used to estimate biomass in units of mass per unit area. Some care should be exercised in using this analysis. Clearly, cover is a two-dimensional measure, while biomass is a characteristic of a three-dimensional mass. Thus, the relationship between cover and biomass will depend on factors such as the form of the vegetation (e.g., flat versus erect), the developmental stage (young versus mature), and the health of the vegetation (thin versus robust at the same percent cover). Different equations should be developed for species that have different forms or for the same species that is in different stages of its life cycle. In some cases, the relationship between cover and biomass may not be linear, so nonlinear (power) functions should be considered.

Other Analyses of Biomass. The same general approach used to estimate macrophyte or bryophyte biomass can be used for more sophisticated analyses as well. Once an estimate of biomass has been obtained as described above, subsamples of the dried biomass

material can be stored for later analysis of any other constituents that might be of interest (e.g., carbon, nitrogen, phosphorus, trace elements). For example, given an estimate of the biomass (g/m^2) and nitrogen content (mg N/mg dry mass) in a dry tissue sample, it is an easy matter to calculate the total nitrogen (TN) mass in a unit of stream area as:

$$TN \ (mg\,N/m^2) = Dry \ biomass \ (g/m^2) \times Nitrogen \ content \ (mg\,N/g \ dry \ biomass) \quad (18.5)$$

III. SPECIFIC METHODS

A. Field Equipment — General Comments

The equipment required to make the field measurements described here is relatively simple (see Section V below for a specific list). One or two items will be particularly useful and can be constructed from materials that can be obtained easily from local suppliers. For both the visual estimation and quantitative point transect approaches to define cover or abundance, a *view scope* will be a useful aid to clearly see the bottom through the surface of the stream. A view scope is simply an opaque box or tube, open on the top and with a clear bottom. A view scope can be purchased from recreational fishing supply stores or can be constructed from a short (25 cm long) section of wide (25 cm diam) PVC pipe that has a piece of Plexiglas sealed on the bottom. Never use glass or other easily breakable materials. Some sort of handle fixed to the PVC tube will make it easier to hold the view scope steady in the stream. If the point transect method will be used, it is helpful to affix a small dot to the center of the view scope bottom to act as sighting target.

A *quadrat* to measure biomass can be constructed from 3/4″ (2 cm) diameter PVC tubing and 90-degree elbow fittings used for household plumbing. Square quadrats that are 25 to 71 cm on a side (0.0625 to 0.50 m^2) are a convenient size for most stream work. Before final assembly of the quadrat frame, it is useful to drill a few small holes in the tubing to allow water to fill and drain easily and to insert a piece of rebar or other heavy rod in the tubes that make up the 4 sides in order to weigh the quadrat down in the stream so that it does not float away.

B. Basic Method: Estimating Biomass from Abundance Data

In this exercise you will (1) develop a relationship between species-specific biomass and cover using the visual plot estimation technique and then, using that relationship, you will (2) estimate macrophyte biomass levels more extensively based on the point transect method. Table 18.3 shows how the biomass-cover data might be organized. Table 18.4 shows how the point transect data might be organized. Further considerations of the example data in these tables are offered in the table legends.

The focus of this exercise will be to estimate biomass in a single stream reach. However the same general approach could be used to compare several stream reaches or could be extended to estimate the total standing stock of key nutrients in plant biomass (e.g., carbon, nitrogen, or phosphorus). While biomass could be measured directly by the harvesting technique, this method is obviously destructive. By relating the direct harvesting method to

					Mass (g dry weight)			Moss Biomass[4]
		Location[1]	Cover[2]				Moss Biomass[4]
Species	**Reach**	**(km)**	**(%)**	**Bag[3]**	**Bag + Moss**	**Moss Alone**	**(gdw/m²)**
Hygrohypnum	Fertilized	2.0	90%	8.871	37.456	28.585	285.9
Hygrohypnum	Fertilized	2.0	75%	8.527	21.217	12.690	126.9
Hygrohypnum	Fertilized	2.0	15%	8.860	16.795	7.935	79.4
Hygrohypnum	Fertilized	2.0	35%	8.858	23.348	14.490	144.9
Hygrohypnum	Recovery	0.95	55%	8.902	19.184	10.282	102.8
Hygrohypnum	Recovery	0.95	95%	8.851	35.335	26.484	264.8
Hygrohypnum	Recovery	0.95	25%	8.866	22.086	13.220	132.2
Hygrohypnum	Recovery	0.95	15%	8.839	16.521	7.682	76.8
Schistidium	Fertilized	2.0	30%	8.836	39.506	30.670	306.7
Schistidium	Fertilized	2.0	20%	8.860	31.013	22.153	221.5
Schistidium	Fertilized	2.0	15%	8.582	10.508	1.926	19.3
Schistidium	Fertilized	2.0	10%	8.732	11.289	2.557	25.6
Schistidium	Recovery	0.95	20%	8.806	24.990	16.184	161.8
Schistidium	Recovery	0.95	30%	8.810	44.060	35.250	352.5
Schistidium	Recovery	0.95	2%	8.820	9.269	0.449	4.5
Schistidium	Recovery	0.95	15%	8.830	16.734	7.904	79.0
Schistidium	Reference	0.05	20%	8.786	18.127	9.341	93.4
Schistidium	Reference	0.05	15%	8.807	15.964	7.157	71.6
Schistidium	Reference	0.05	10%	8.840	11.246	2.406	24.1
Schistidium	Reference	0.05	5%	8.813	10.449	1.636	16.4

TABLE 18.3 Moss Biomass and Cover Data from the Kuparuk River, Alaska, on June 30, 1999. *Hygrohypnum* spp. and *Schistidium agasizii* are two important species found in this river. The two groups differ substantially with respect to their physical form and ecophysiological behavior. In this case, samples were taken in three different reaches of the Kuparuk River: (1) an upstream reference reach; (2) a downstream reach that had been fertilized with low-levels of phosphorus annually since 1983; and (3) a recovery reach between in which fertilization ceased in 1996. In this case, plots (0.1 m² quadrats) were selected on purpose to represent the observed range of visual cover classes in each of the three experimental reaches. Note that for ecophysiological reasons, *Hygrohypnum* spp. do not exist in the unfertilized reference reach. For more information see Arscott *et al.* (1998 and 2000).

[1] *Location is relative to a fixed arbitrary 0-km point on the river. The 0-km point is upstream of all of the sampling points. The location value for the sampling points increases downstream. Thus, the fertilized reach sampling points are furthest downstream at 2 km.*

[2] *Cover within the quadrat was estimated visually by two people before the qaudrat was sampled. The recorded value is the consensus estimate.*

[3] *This is the weight of the dry, empty paper bag. Note that weights in this and the next two columns were obtained from a balance capable of weighing to ±0.001 g.*

[4] *Moss biomass is expressed as grams dry weight (gdw) per square meter, based on the dry mass of moss in each bag and the quadrat area of 0.1 m² from which the moss was collected. Note that the least significant digit in these estimates is ±0.1 gdw/m².*

the point transect method, it is possible to perform repeated, nondestructive sampling. This can be important when the target species is sparsely distributed or rare.

In the steps that follow it is advisable to avoid lengthy delays (weeks) between completing the visual plot estimates and completing the point transect observations, to minimize the effects of exogenous factors (e.g., seasonal plant growth, disturbance from storm

TABLE 18.4	Example Summary of Point Transect Data from a Single Station in the Kuparuk River, Alaska, in 2003. In this case, eight observable classes were categorized. At an interval of 10 cm along five transects across the river, the dominant class of cover on the river bottom was selected from among these eight classes. This table is a summary of the number of times each class was observed ("hit") in each transect (top panel) and the percent of total "hits" in each transect represented by each class (bottom panel). The width of each transect is simply the total number of hits per transect, times the observation interval (10 cm in this case). In the suggested protocols, these data would represent a single data point (the mean value) for each class in one reach. At least three similar data points (transect sets) should be collected for each reach type in the study. Verify that the cover of epilithic diatoms (microalgae on top of rocks) is similar to that of *Hygrohypnum* spp. at 47% and 44%, respectively, and that *Schistidium* is absent from this station.

	Number of "Hits" per Class by Transect				
	Transect Number				
Class	**1**	**2**	**3**	**4**	**5**
Epilithic diatoms	38	49	33	24	24
Filamentous alga A	0	0	4	0	0
Filamentous alga B	0	0	0	0	0
Filamentous alga C	0	0	0	2	0
Hygrohypnum	28	22	27	32	41
Schistidium	0	0	0	0	0
Detritus	1	9	11	0	0
Unknown	2	2	1	2	0
Total "hits" per transect	69	82	76	60	65
Width (m)	6.9	8.2	7.6	6	6.5

	Percent of "Hits" per Class by Transect Total				
	Transect Number				
Class	**1**	**2**	**3**	**4**	**5**
Epilithic diatoms	55%	60%	43%	40%	37%
Filamentous alga A	0%	0%	5%	0%	0%
Filamentous alga B	0%	0%	0%	0%	0%
Filamentous alga C	0%	0%	0%	3%	0%
Hygrohypnum	41%	27%	36%	53%	63%
Schistidium	0%	0%	0%	0%	0%
Detritus	1%	11%	14%	0%	0%
Unknown	3%	2%	1%	3%	0%
Sum	100%	100%	100%	100%	100%

events). The steps are organized in a logical chronological order and include practical operational guidance. Refer to the previous section (Section II) for general design and methods considerations.

1. Quickly reconnoiter the stream to get a sense of where and how the macrophytes or bryophytes are distributed. In environments where the plant distribution is patchy, it may be necessary to use a more intensive sampling procedure (see step 6).

2. Select (i.e., *not* randomly) a set of plot locations that represent a range of different cover and biomass conditions for each dominant species in the stream. The range should include areas that appear to have maximum vegetation density, down to areas that have very sparse vegetation. Ideally you should include several replicate plots (at least three) for each cover class in the Braun-Blanquet (or other) scale (Table 18.2) and then harvest the species' biomass in these plots according to the instructions in step 3.

3. Harvest all biomass of the individual species of interest within the quadrat. Macrophytes can be simply uprooted from the sediment. Take care, however, because fine roots might be left behind. If the objective is to obtain total above- and belowground biomass it is important to retrieve as much of the belowground biomass as possible. This is difficult to do and for that reason many researchers have focused on aboveground biomass only. However, belowground biomass of many macrophytes species may equal or exceed (by many times) the above ground biomass. For bryophytes, use a single-edged razor blade to scrape the biomass from rocks. Wear gloves to reduce the risk of cutting yourself with the razor. Any plant whose stem or base lies within the quadrat should be harvested, even if its leaves hang over the quadrat edge. Leaves from plants whose stem or base lie outside the quadrat should not be sampled.

4. Before you start to collect the point transect cover data, identify the key stream sites that you wish to sample. Your sampling plan should include a minimum of three replicates of each type of site that you intend to compare in your study. For example, if you want to compare the bryophyte cover and biomass in the riffles of two different streams, then you should identify at least three riffles in each stream (six total sites). More replicates are desirable in streams with patchy environments.

5. At each site, stretch a field tape across the stream to define a transect and use the view scope to move along the tape at regular intervals to identify the species beneath the target dot on the view scope. Do not "search" for a plant. Rather, record whatever you first see under the target dot. Include "bare" or "unvegetated" as one of your categories. Resist the temptation to record "mixed" scores (i.e., always record the dominant cover). Develop a simple alphanumeric key so that you can efficiently record species without having to write down a lengthy name. It is most efficient if two people work in the field as a team, with one person moving across the stream calling out the observed cover types and one person on the shore to record the observations.

6. At each site, do several transects across the stream, spaced evenly along the site. The spacing between transects may vary from 1 to many meters, depending on the length of the stream feature you intend to examine. As a rule of thumb: if your site is longer than 10× the width of the stream, you might want to consider dividing the site into multiple subsites and then sample only one representative subsite. The sampling interval across each transect may vary from 5 to 50 cm depending on the stream width and the "patchiness" of the plant community. If the stream is wide and plant community is evenly distributed, wider sampling intervals are acceptable. However, for narrow streams or where plant cover is patchy, it would be better to use smaller sampling intervals. As a guide, adjust the number of transects and the sampling interval across each transect so that you obtain at least 250 points for the site. For example, a stream that is 5 m wide, 20 cm intervals will provide 26 points per transect (including 0 and 5 m) and so 10 transects are needed to obtain at

least 250 points. The number of transects per site should never be fewer than 5 because the variation among transects can be substantial. In wide streams it is therefore better to decrease the number of points per transect (increase the sampling interval across the transects) than to decrease the number of transects below 5. For more rigorous work it would be better to collect 500 to 1000 points at a site, decreasing the sampling interval and increasing the number of transects. The degree of accuracy you desire will have to be balanced against the amount of effort you can expend to collect these data. An experienced team can complete 5 transects at a site in 30 to 60 min. The same sampling interval should be used for all transects at a site and, to the degree possible, for all sites used in a particular comparison.

7. In the laboratory, process and dry the field samples as described in the general instructions in Section IIC (Laboratory Processing). Separate paper bags should be used for each species, cover class, and replicate.

8. Once the biomass samples have dried, weigh them and plot the measured biomass (g/m^2) versus the observed cover in the quadrats. Use a linear or nonlinear regression to determine the best-fit equation for this relationship. Calculate and compare separate curves for each species. See Table 18.3 for an example data set and guidance.

9. Calculate percent cover from the point transect data as described in Section IID (Data Reduction and Analysis) above. See Table 18.4 for an example data set and guidance.

10. Use the regression equation devised in step 7 above to convert the percent cover data as calculated from the point transect data, to biomass at all sites. See Table 18.5 for an example data set and guidance.

TABLE 18.5	Conversion of Point Transect Cover Data to Biomass Data Using the Biomass Cover Regression. Data were collected in 2001 in the Kuparuk River, Alaska, at three different times in the season (early, middle, and late). All stations were in the fertilized reach. The cover data are from point transect collections. Using the data in Table 18.3, the regression for *Hygrohypnum* biomass on cover (by the plot method) is biomass = ($274 \times$ % cover) for the fertilized reach. (Why shouldn't you use all of the *Hygrohypnum* data in Table 18.3?) The intercept in this regression has been forced through zero. (Why?) Using this regression, it is possible to calculate the biomass of *Hygrohypnum* that was present at each reach on each date. Does there appear to be much of a difference in biomass in this reach among the three sampling dates?

Season	Station (km)	Cover (%)	Biomass (gdw/m²)
Early	1.95	22%	61
Early	2.1	85%	232
Early	3.0	48%	132
Middle	1.95	30%	82
Middle	2.1	82%	223
Middle	3.0	61%	168
Late	1.95	36%	99
Late	3.0	64%	176

C. Advanced Method: Effect Analysis of Flow Regime on Community Structure

In this more advanced exercise you will study the effect of flow regime (frequency and duration of high flow events) on the distribution and abundance of stream macrophytes or bryophytes. The effect of any other physical or chemical factor could be evaluated in a similar way.

1. Identify at least two streams in the same geographic region that have different flow regimes. It would be better to have several streams that provide a range of flow characteristics or that represent distinctly different types of flow regime. For example, streams with relatively stable flow regimes are usually present downstream of lakes that dampened the effect of individual rainfall events and in areas where streams are almost exclusively fed by groundwater springs. Most other unregulated streams have flow regimes that vary strongly in response to rainfall events. Use available resources (e.g., stream discharge records, maps, local knowledge) to identify stream reaches that have different flow regimes and that also have at least some macrophyte or bryophyte communities present.

2. For each of the selected streams, choose at least three representative stream reaches without shading (50–100 m) in which to do the assessments. The stream reach might be physically heterogeneous or homogenous depending on what is representative for that stream type. All reaches should be similar in terms of habitat conditions other than flow regime (e.g., depth, substrate type, turbidity, shading, soil properties, geological and geographical area). Note that, strictly speaking, the "replicable unit" in this study is the stream flow type and not the individual measured stream reach, which is only a subsample. If you only measure two stream flow types (e.g., one with a fluctuating flow regime and one with a stable flow regime), it is impossible to say whether any differences you observe are due to the difference in flow regime or to some other unmeasured factor, even when multiple reaches are measured in each stream type. Simple comparisons of, say, three reaches in one stream with three reaches in another different stream may be useful and informative. However, for the most rigorous results, it is usually better to invest resources (time and effort) in more true replicates (stream types) and fewer subsamples (reaches) than in more subsamples and fewer replicates.

3. Identify different habitat types (e.g., riffle, run, pool; see Chapter 2) for each stream reach. Randomly locate at least 10 observation plots (e.g., 0.5×0.5 m) in each habitat type by tossing a quadrat into the habitat area.

4. For each plot record the presence of each species and estimate its abundance using the Braun-Blanquet scale (Table 18.2). Collect specimens for any individuals you can not readily identify so that you can make voucher specimens for later examination.

5. Other important characteristics of your observation plots may also differ. At each site record any of these characteristics that you think might be important (e.g., water depth, current velocity, substrate characteristics, canopy cover).

6. Use the methods described in Section IID to calculate species abundance, richness, diversity, and composition for the 10 most common species in each stream reach.

7. Several approaches can be used to test whether there are significant differences in community measures at the reach scale among stream types. Measures such as species richness, vegetation frequency, species frequency, species diversity, and evenness can be tested directly (e.g., Kent and Coker 1995, Miller and Ricklefs 1999,

Grime 2001, Townsend *et. al.* 2002). Species composition of different stream types can be compared by testing the mean occurrence of the ten most abundant species in the observation plots of the three reaches in each stream type. If only two different stream types were observed, use "two-sample" tests, as described by Zar (1999). If the data are normally distributed, a simple Student's t-test can be used. If the data are not normally distributed or the distribution is unknown, use a Mann-Whitney U-test. If a range of stream types was observed, rather than two distinct classes, a regression analysis might be appropriate (Zar 1999). Prior to doing a regression analysis it is usually helpful to plot the dependent variables (i.e., community measures such as species abundance, richness, or diversity) versus the independent variable (flow frequency or probability in this case). This exploratory analysis of the data can be helpful in deciding whether a linear or non-linear regression analysis is most appropriate for the data. See the example of a fictional data set in Tables 18.6–18.8, corresponding to the exercise outlined here.

8. The basic approach outlined here could be expanded easily to explore more complex associations in streams. For example, you could sample invertebrate or algal epiphyte populations living on macrophytes or bryophytes in the different stream flow types. This might involve looking at the number of individuals per plant dry mass or the plant surface area using information and approaches discussed in other chapters of this book.

TABLE 18.6	Example Layout of a Table Used to Summarize Data for the Advanced Method Outlined in Section IIIC. Data for this exercise can be found on the website for this book. More or fewer species could be added to each stream type and habitat combination. The sampling plan could be modified by having more reaches (m) or more plots in each reach (n).

			Occurrence of Indicated Species by Plot in Each Reach											
			Reach 1				Reach 2				Reach m			
Stream Type	Habitat	Species	1	2	...	n	1	2	...	n	1	2	...	n
Stable	Riffle	Spp1												
		Spp2												
		etc.												
	Run	Spp1												
		Spp2												
		etc.												
	Pool	Spp1												
		Spp2												
		etc.												
Disturbed	Riffle	Spp1												
		Spp2												
		etc.												
	Run	Spp1												
		Spp2												
		etc.												
	Pool	Spp1												
		Spp2												
		etc.												

| | | | TABLE 18.7 | Example Layout of a Table Used to Summarize Physical Parameters (here, mean water depth and current velocity) by Stream Reach in Two Different Stream Types with Three Different Habitats, as in the Advanced Method in Section IIIC and Table 18.6. Data for this exercise can be found on the website for this book. In different experimental designs, different factors might be used. This table could be further altered by including more or fewer reaches (m) and by varying the number of observation plots in each reach (n). |

TABLE 18.7 Example Layout of a Table Used to Summarize Physical Parameters (here, mean water depth and current velocity) by Stream Reach in Two Different Stream Types with Three Different Habitats, as in the Advanced Method in Section IIIC and Table 18.6. Data for this exercise can be found on the website for this book. In different experimental designs, different factors might be used. This table could be further altered by including more or fewer reaches (m) and by varying the number of observation plots in each reach (n).

			Physical Parameters by Plot in Each Reach
			\multicolumn{4}{c}{Reach 1}	\multicolumn{4}{c}{Reach 2}	\multicolumn{4}{c}{Reach m}
Stream Type	Habitat	Parameter	1	2	...	n	1	2	...	n	1	2	...	n
Stable	Riffle	Water depth (m) Current velocity ($\mathrm{m\,s^{-1}}$)												
	Run	Water depth (m) Current velocity ($\mathrm{m\,s^{-1}}$)												
	Pool	Water depth (m) Current velocity ($\mathrm{m\,s^{-1}}$)												
Disturbed	Riffle	Water depth (m) Current velocity ($\mathrm{m\,s^{-1}}$)												
	Run	Water depth (m) Current velocity ($\mathrm{m\,s^{-1}}$)												
	Pool	Water depth (m) Current velocity ($\mathrm{m\,s^{-1}}$)												

TABLE 18.8	Species Richness, Shannon Diversity, and Species Evenness for Each of the Surveyed Reaches Based on Data in Table 18.6. Is there any difference in species richness, Shannon diversity and evenness between habitats in each stream type? (Compare riffle, run and pool in each stream type.) Is there any difference between habitats in the two stream types? (Compare, for example, riffles in stable and disturbed streams.) What conclusions might you make by comparing the results in this table to the physical characteristics noted in Table 18.7?				

Stream Type	Habitat	Vegetation Parameter	Reach 1	Reach 2	Reach 3
Stable	Riffle	Species richness	2	2	2
		Shannon diversity	0.69	0.69	0.69
		Species evenness	1.00	0.99	1.00
	Run	Species richness	5	4	5
		Shannon diversity	1.54	1.36	1.55
		Species evenness	0.96	0.98	0.97
	Pool	Species richness	4	5	5
		Shannon diversity	1.36	1.54	1.51
		Species evenness	0.98	0.95	0.94
Disturbed	Riffle	Species richness	0	0	0
		Shannon diversity	0	0	0
		Species evenness	0	0	0
	Run	Species richness	2	2	3
		Shannon diversity	0.53	0.35	0.95
		Species evenness	0.76	0.50	0.86
	Pool	Species richness	3	3	2
		Shannon diversity	0.57	0.85	0.38
		Species evenness	0.52	0.77	0.54

IV. QUESTIONS

1. How would you expect the three-dimensional structure and distribution of a macrophyte or bryophyte species to affect the relationship between biomass and cover?
2. Would you expect cover-biomass relationships to be readily transferable from one stream to another? If not, why not? Under what conditions might this be acceptable?
3. In the Advanced Exercise, what other factors could cause differences in community measures, other than the focal factor of flow regime? Are these factors independent or dependent in any way on flow regime?
4. Is there a relationship between plant form (e.g., upright or lying flat) and the average flow regime that plant prefers?
5. How many plots or points would you have to observe before you encountered all of the species in your study site(s)?
6. What is the relationship between macrophyte or bryophyte cover and insect abundance in your streams?
7. Over a season, does cover change as much as biomass?

V. MATERIALS AND SUPPLIES

Field Gear

    50-m field tape
    View scope
    Quadrat (for biomass sampling only)
    Single-edged razor blades (for biomass sampling only)
    Plastic, Ziplock storage bags, 1 gal (or 4 L, for biomass sampling only)
    Tennis shoes for wading (felt bottom boots are better)
    Waders (if the water is cold)
    Notebooks, pencils (not pens), indelible marker
    Field identification books (optional)
    Hand lens (optional)

Laboratory Gear

    Manila folders or bond paper for voucher specimens
    Small (10 L) plastic wash tub
    Paper bags (for biomass sampling only)
    Drying oven (or fan-bake conventional oven)
    Balance with 0.1% to 1% accuracy for the median sample mass (i.e., ± 0.1 g
        for a 10 g sample gives 1% accuracy)
    Microscopes, ideally both dissecting and compound, to examine fine structures
        of macrophytes and bryophytes for taxonomic identification

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APPENDIX 18.1
Field Key to Genera of Common North American Stream Bryophytes

This key will require a hand lens and a careful examination in good light. For more accurate identification or identification to the species level, a microscope is needed, plus access to a good key such as *Mosses of Eastern North America* (Crum and Anderson 1981). Other field guides and keys will provide additional useful infromation and illustrations; e.g., *How to Know the Mosses and Liverworts* (Conard and Redfearn 1979) and *Mosses, Lichens, & Ferns of Nortwest North American* (Vitt *et al.* 1988). Key technical words are defined briefly in the following key.

Plants **dorso-ventrally** differentiated (liverworts – have a top/bottom orientation)
  Plants **thalloid** (thallose liverworts – like a branched blade)
    Thallus with conspicuous **pores**; surface usually with distinct **polygons**
      Thallus with **raised pores** resembling volcanoes; midrib
        lacking . . . . *Conocephalum conicum* (Fig. 1)
      Thallus thick, with **depression** resembling a **midrib**; branching **dichotomous**
        (forked in pairs) . . . . *Marchantia* (Fig. 2)
    Thallus **lacking** conspicuous **pores**; surface lacking polygons,
      with thickened middle and thin margins; **midrib** faint or lacking; branching
      irregular . . . . *Pellia* (Fig. 3)
  Plants leafy; leaves in two rows (leafy liverworts)
    Leaves round, with conspicuous **underlobes** (fold of leaf on
      underside); underleaves (along stem) conspicuous; on trees or rocks, usually at
      high water line . . . . *Porella* (Fig. 6)
    Leaves **lacking underlobes**
      Leaves strongly **folded**, with smaller lobe on top . . . . *Scapania* (Fig. 4)
      Leaves not folded

Leaves oblong; leaf base **dorso-ventrally decurrent** (curved down
   stem to form edge on both upper and lower side), causing leaf to
   appear gathered at base .... *Plagiochila* (Fig. 5)

Leaves variously rounded to oblong .... *Chiloscyphus,*
   Jungermannia, Marsupella, Nardia (Figs. 7, 8)

Plants **upright** or **similar on top and bottom**, not dorso-ventrally (top/bottom)
  differentiated (mosses)

  Branches clumped in groups of three or more, usually crowded at apex
    to form a **capitulum** (head); cells of two types (dead cells filled with water and
    green cells) ....*Sphagnum* (Fig. 9)

  Branches single; lacking dead cells filled with water

    Plants with two rows of leaves or appearing so; leaves with **costa** (central rib)

      Leaf fitting into **pocket** of leaf below; leaves often oblong, rounded; branches flat
      ....*Fissidens* (Fig. 10)

      Leaf **lacking pocket**; leaves lanceolate (long and tapering to point like a sword),
      pointed ....*Leptodictyum* (Fig. 15)

    Plants with more than two rows of leaves; leaves with or without **costa** (central rib)

      Leaf margins rolled under

        Leaves often **contorted** when dry; cells having **papillae** (microscopic
        projections); can make rock formations due to carbonate
        deposits ....*Didymodon* (Fig. 14)

        Leaves **not contorted** when dry; cells often **lacking papillae**; plants often blackish
        ....*Schistidium* (Fig. 11)

      Leaf margins flat

        Plants with leaves in three rows

          Leaves lacking **costa** (central rib); leaf often keeled like
          a boat ....*Fontinalis* (Fig. 16)

          Leaves with **costa** (central rib)

            Leaves curved, **acuminate** (long tapering point)......*Dichelyma* (Fig. 17)

            Leaves straight, **acute** (sharp point) ....*Brachelyma* (Fig. 18)

        Plants with more than three rows of leaves

          Plants blackish ....*Schistidium, Scouleria* (Figs. 11, 13)

          Plants green, reddish, or yellowish

            Leaves with thick **border** of 2-3 layers of linear
            cells ....*Platylomella, Limbella* (Fig. 26)

            Leaves **lacking border**

              Leaves **keeled** (folded like keel of a boat), tips
              rounded ....*Racomitrium* (Fig. 12)

              Leaves not **keeled**, tips pointed

                Leaves **ovate** (shaped like egg, but may be pointed)

                  Costa (central rib) short or double; leaves often curved
                  toward tip ....*Hygrohypnum* (Fig. 19)

                  Costa reaching near leaf tip, single; leaves not curved

                    Leaf blade **decurrent** (edges extending down stem at
                    insertion) ....*Brachythecium rivulare* (Fig. 20)

                    Leaf blade **not decurrent**

                      Leaves deeply concave; stems with leaves appear round
                      in cross sec (**julaceous**) ....*Scleropodium* (Fig. 27)

                      Leaves flat or slightly concave, not cup-like

Leaf **tips blunt** or acute; apical cells of branch
 leaves short, unlike median cells ....
 ....*Platyhypnidium riparioides* (Fig. 21)
Leaf **tips elongate-pointed** (acuminate); apical
 cells of branch leaves elongate, like median
 cells .... *Brachythecium* (Fig. 28)
Leaves **lanceolate** (long and tapering to tip like sword) to long lanceolate,
 possessing a distinct **costa** (central rib)
Leaves strongly **curved**, at least at stem and branch tips
 Paraphyllia (filaments among leaves) present between
 leaves .... *Cratoneuron commutatum* (Fig. 22)
 Paraphyllia absent between leaves ... ... *Drepanocladus* (*s.l.*) (Fig. 23)
Leaves more or less straight
 Alar cells (cells at leaf base margins) **inflated** in well-marked groups
 ....*Cratoneuron filicinum* (Fig. 24)
 Alar cells not inflated
 Costa strong, remaining when blade eroded away; leaf blade
 never folded like Japanese fan ...... *Hygroamblystegium* (Fig. 25)
 Costa thin, not remaining when blade eroded away; leaf blade
 often folded like Japanese fan ....*Brachythecium* (Fig. 28)

Figure legends and photo credits for Appendix 18.1

1. *Conocephalum conicum* showing thallus with polygons and raised pores. Photo by Janice Glime.
2. *Marchantia polymorpha* showing pores, groove with rib, and gemmae cups. Photo by Janice Glime.
3. *Pellia endiviifolia* showing thallus with reproductive structures. Photo by Janice Glime.
4. *Scapania nemorea* showing folded leaf. Photo by Michael Lüth. Photo by Janice Glime.
5. *Plagiochila porelloides* showing drying leaf that appears to be gathered at base. Photo by Janice Glime.
6. *Porella pinnata* showing round leaves; plants typically found on trees at high water level. Photo by Janice Glime.
7. *Chiloscyphus pallescens* showing thin, rounded leaves. Photo by Michael Lüth.
8. *Marsupella aquatica* branch showing overlapping leaves. Photo by Michael Lüth.
9. *Sphagnum fallax* showing terminal capitulum with crowded branches. Photo by Janice Glime.
10. *Fissidens* showing flat branch and leaves with strong *costa* and pocket. Photo by Michael Lüth.
11. *Schistidium rivulare* showing growth habit. Photo by Michael Lüth.
12. *Racomitrium aquaticum* showing closely overlapping leaves and dark color. Photo by Michael Lüth.
13. *Scouleria aquatica* showing blackish color. Photo by Janice Glime.
14. *Didymodon tophaceus* showing accumulation of $CaCO_3$ at base. Photo by Michael Lüth.
15. *Leptodictyum riparium* showing flattened leaves and branches. Photo by Michael Lüth.

16. *Fontinalis squamosa* showing leaves with no rib and in three rows. Photo by Michael Lüth.
17. *Dichelyma pallescens* growing on a low branch that is submerged when water is high. Photo by Janice Glime.
18. *Brachelyma subulatum* hanging from tree and typically submerged when water is high. Photo by Janice Glime.
19. *Hygrohynum ochraceum* showing curved leaves. Photo by Michael Lüth.
20. *Brachythecium rivulare* showing shiny leaves. Photo by Michael Lüth.
21. *Platyhypnidium riparioides* showing ovate leaves that are not curved. Photo by Michael Lüth.
22. *Cratoneuron commutatum* var. *falcatum* showing curved leaves and tips. Photo by Michael Lüth.
23. *Drepanocladus fluitans* showing lanceolate and curved leaves. Photo by Michael Lüth.
24. *Cratoneuron filicinum* showing straight leaves and branched growth habit. Photo by Michael Lüth.
25. *Hygroamblystegium fluviatile* showing tight growth habit. Photo by Michael Lüth.
26. *Limbella* branch and *Platylomella tricostatum* showing thickened leaf margins. Photos by David Wagner and Masanobu Higuchi.
27. *Scleropodium obtusifolium* showing concave leaves. Photo by Paul S. Wilson.
28. *Brachythecium geheebii* showing leaf rib (costa) and folds (plications). Photo by Michael Lüth.

leaf pocket
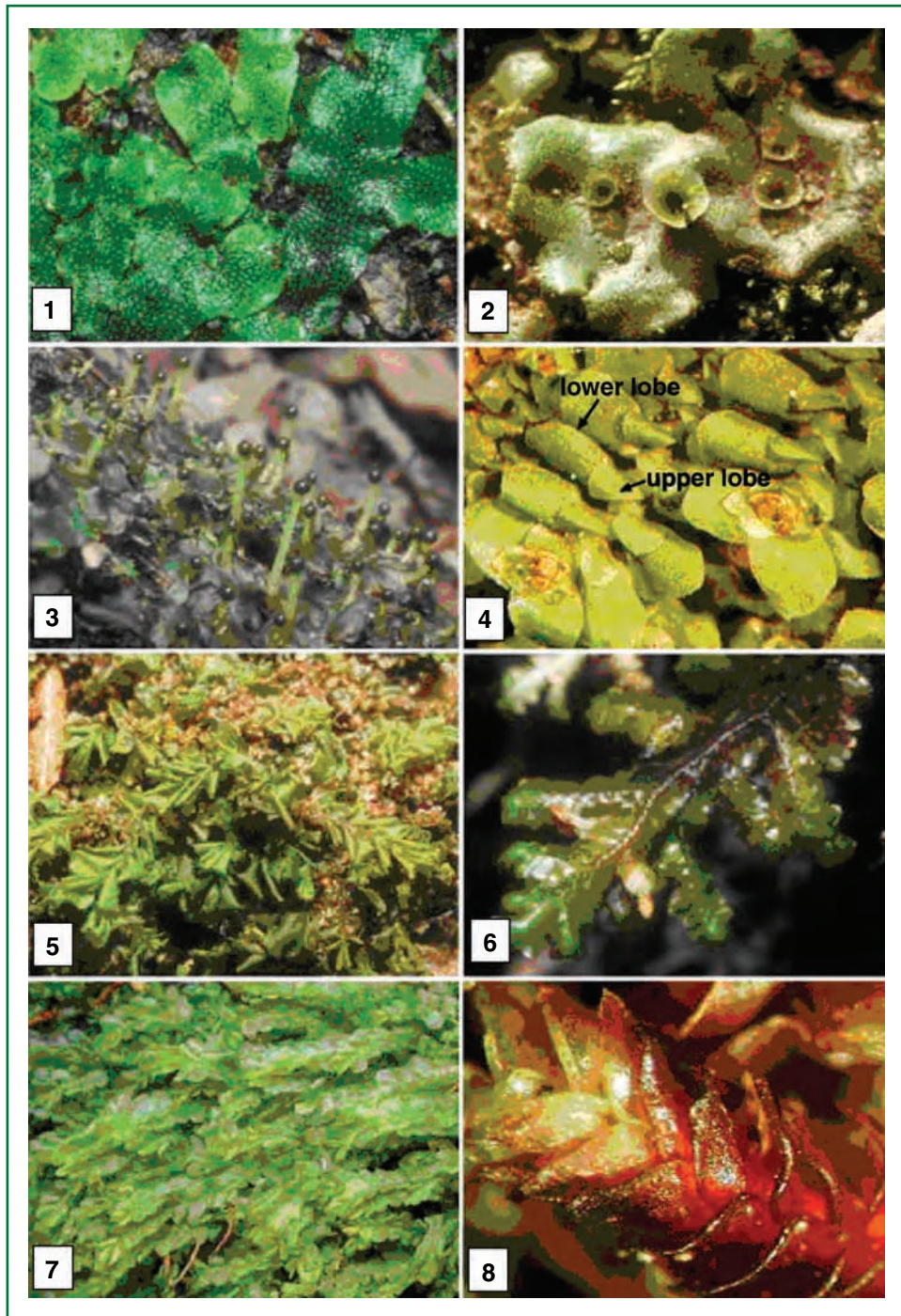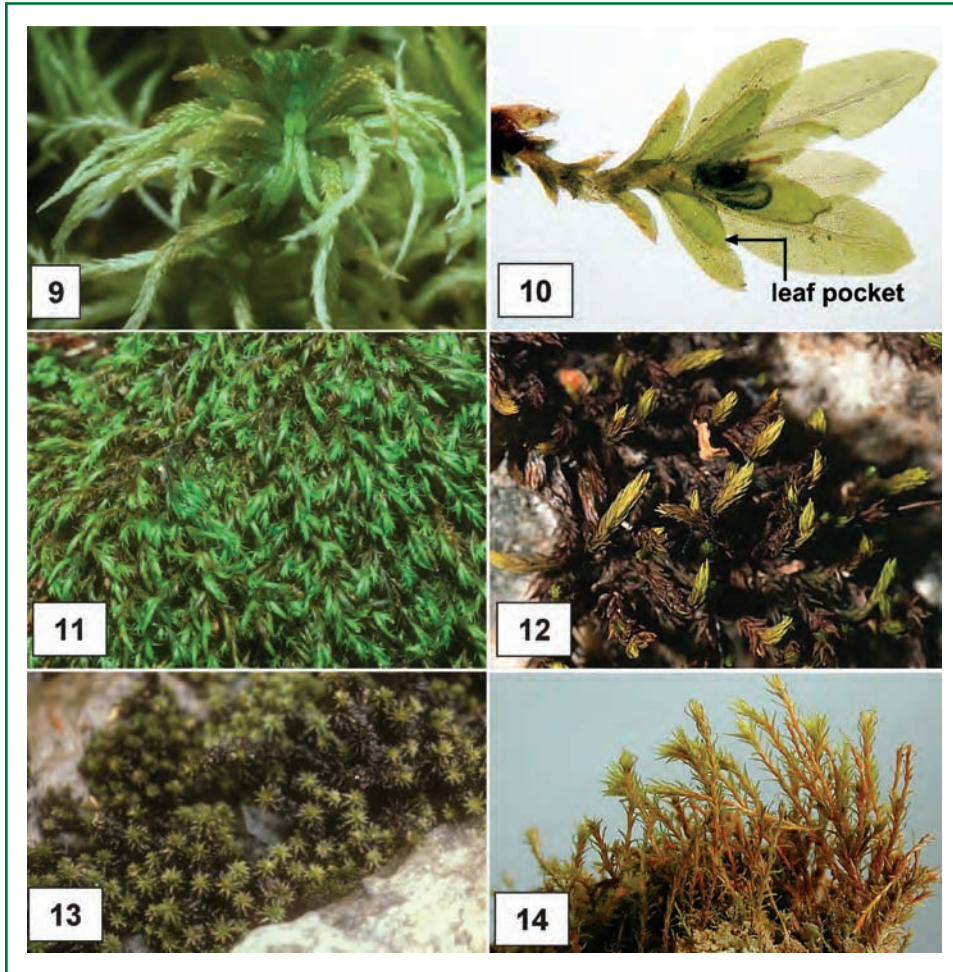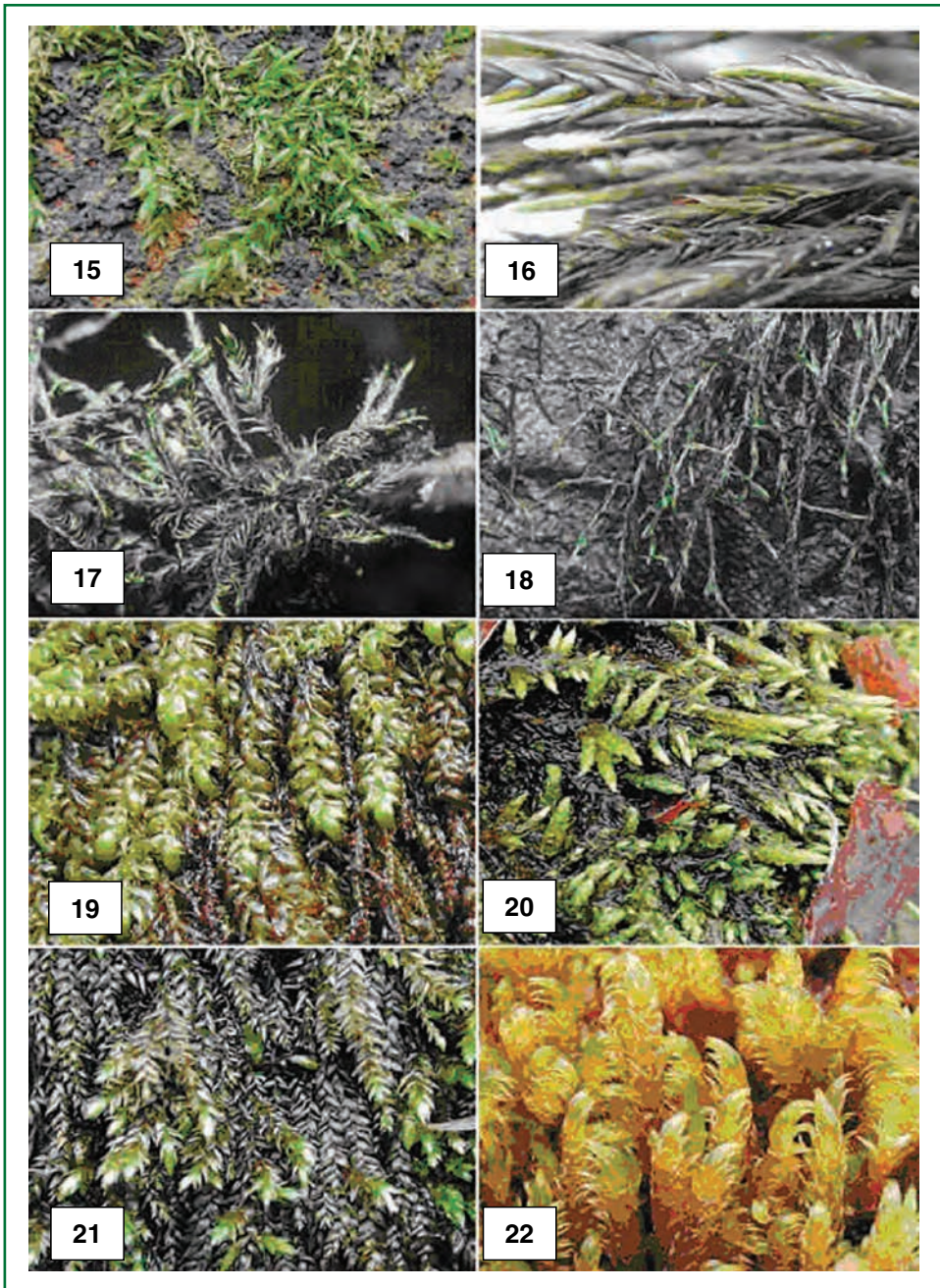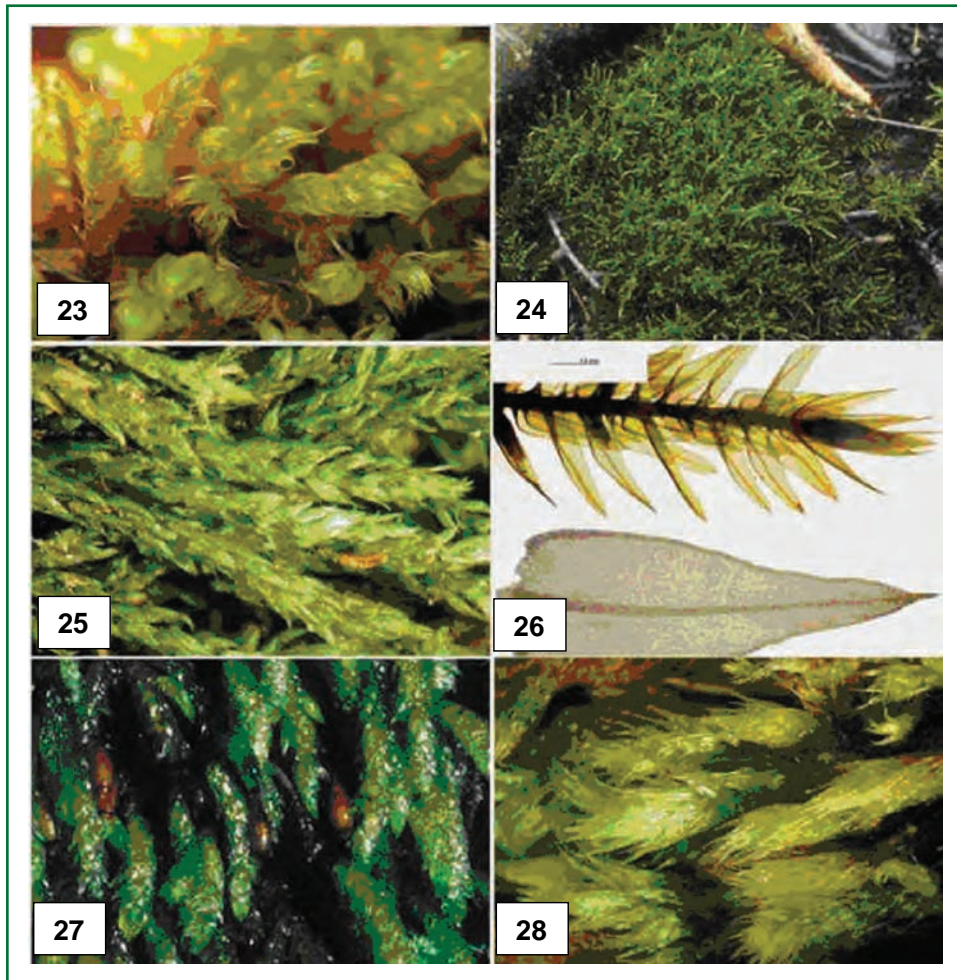
Meiofauna

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I. INTRODUCTION

The *meiofauna* are defined as those benthic animals that pass through a 500-μm sieve but are retained on a 40-μm sieve (Fenchel 1978, Higgins and Thiel 1988). The past decade has seen a huge increase in interest in this fauna, which often dominates benthic animal communities in terms of numbers and species richness, and plays important roles in community and ecosystem processes (Robertson *et al.* 2000, Rundle *et al.* 2002). Stream meiofauna communities are usually dominated by rotifers, harpacticoid and cyclopoid copepods, young chironomids, naidid and enchytraeid oligochaetes, and nematodes (Whitman and Clark 1984, Pennak and Ward 1986, Strayer 1988, Palmer 1990), but often also contain flatworms, gastrotrichs, tardigrades, cladocerans, ostracods, mites, and the young of various insects (Figure 19.1). Meiofauna are often classified as *permanent* (species spending their whole lives as meiofauna) or *temporary* (animals such insects that start off as meiofauna but grow into macrofauna). Local communities typically contain hundreds of species (Robertson *et al.* 2000), some of which may be new to science. Indeed, the recent discovery of a new meiofaunal phylum (Micrognathozoa) in a Greenland stream suggests that their contribution to freshwater diversity may yet be more substantial (Funch and Kristensen 2002).

Interstitial meiofauna live between grains of sand and typically are small and worm-shaped. Many interstitial species have adhesive organs for attaching to sand grains. *Burrowing meiofauna* live in fine sediments and often have robust bodies for pushing aside mud and silt. *Epibenthic meiofauna* live on the streambed or on wood, leaves, or plants. These typically are the largest members of the meiofauna and often are good swimmers. Like other animals, meiofauna are patchy in distribution (e.g., Rouch 1991), and recent reviews have attempted to unravel the main factors responsible for this small scale patchiness (Robertson 2000, Swan and Palmer 2000, Silver *et al.* 2000, 2004),
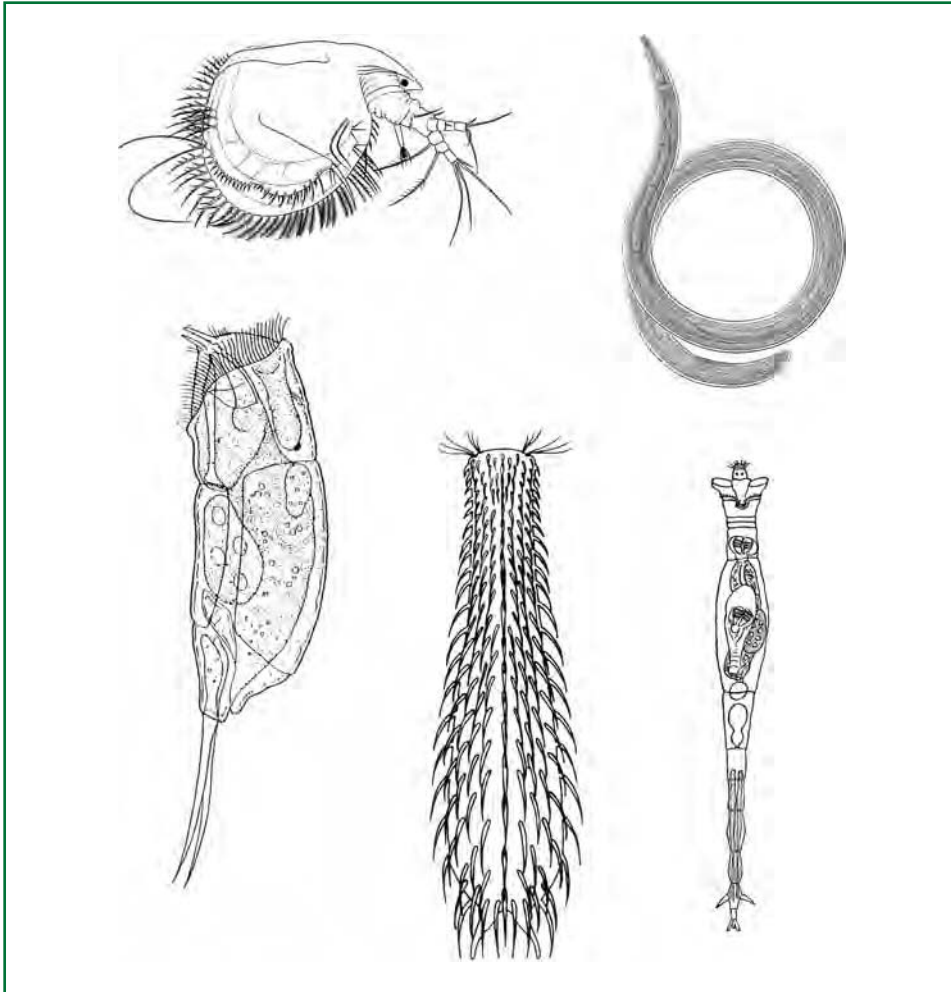
FIGURE 19.1 A few common kinds of freshwater meiofauna. Clockwise from upper left: a benthic cladoceran (*Ilyocryptus sordidus*), a free-living nematode (*Dorylaimus stagnalis*), a bdelloid rotifer (*Rotaria tridens*), a gastrotrich (*Chaetonotus* sp.), and a ploimate rotifer (*Cephalodella* sp.). Animals are not all drawn to the same scale. Modified from Strayer (1985, 2004) after various sources.

including surface-subsurface water dynamics (Boulton *et al.* 2002). At the same time, it is also clear that large-scale phenomena such as plate tectonics and glaciation have an influence on trends in regional diversity and, ultimately, local diversity and community dynamics (Strayer and Reid 1999, Rundle *et al.* 2000, 2002). The role that dispersal plays in driving meiofaunal distribution patterns and, indeed, the ways in which meiofaunal taxa disperse represent exciting areas for future study (Robertson 2002).

Both biotic and abiotic factors likely play a role in regulating the distribution and abundance of stream meiofauna at smaller scales. Predation by fish and macrobenthos has been shown to structure some marine meiobenthic assemblages (Coull 1990) and may also be important in freshwater systems (Shofner 1999, Schmid-Araya and Schmid 2000). Dissolved oxygen, organic matter content, and water flow may be the most important

abiotic factors regulating meiofaunal populations at small scales in stream sediments (Swan and Palmer 2000). Most stream meiofauna are obligate aerobes, and several studies have found a correlation between oxygen and meiofaunal populations (Boulton *et al.* 1991, Rouch 1991). Perhaps in response to gradients of dissolved oxygen, most meiofauna are found in the top few centimeters of sediment (Danielopol 1976, Coull 1988, Palmer 1990). During spates, however, the upper layers of sediment may be denuded of meiofauna as substratum is eroded and the animals are swept downstream (Marmonier and Creuzé des Châtelliers 1991, Palmer *et al.* 1992, Robertson *et al.* 1995).

Because dissolved oxygen and interstitial water flow are influenced by sediment grain size, the latter may be a good predictor of meiofaunal abundance and composition (Coull 1988, Pennak 1988, Ward and Voelz 1990). Gravel harbors an abundant and diverse meiofauna, particularly rotifers, copepods, and tardigrades. Sands and silts are inhabited chiefly by oligochaetes, chironomids, and nematodes. Other physico-chemical factors (e.g., temperature and pH) may also be important regulators at regional scales (Hummon *et al.* 1978, Rundle and Ramsay 1997). Meiofauna also are fairly sensitive to various pollutants, and may be useful as indicator species (Coull and Chandler 1992, Burton *et al.* 2002).

Meiofauna show marked seasonality in reproduction and abundance. In most temperate streams, meiofaunal populations reach peak abundances in late spring through early fall, up to 6,000,000/m^2 (Danielopol 1976, Hummon *et al.* 1978, Strayer 1988, Strayer and Bannon-O'Donnell 1988, Palmer 1990, Shiozawa 1991, Suren 1992). Meiofauna may constitute >95% of the benthic animals in most streams and may be energetically important, although information on their functional roles is patchy and estimates of their functional importance vary (Hakenkamp and Morin 2000, Hakenkamp *et al.* 2002). Nevertheless, some studies (e.g., Poff *et al.* 1993) suggest that the meiofauna may be responsible for most of the benthic respiration in some rivers, and the activities of stream meiofauna have been shown to affect microbial communities and detrital dynamics (Perlmutter and Meyer 1991, Borchardt and Bott 1995). Finally, recent detailed studies of the role of meiofauna in stream food webs suggest that they dramatically increase web complexity and substantially alter food web metrics such as connectance (Schmid-Araya *et al.* 2002a, b).

The objectives of this chapter are to introduce the meiofauna as a taxonomic and ecological group and to introduce methods used in the study of stream meiofauna. First, we provide an overview of field sampling and the basic methods for the observation, identification, and quantification of meiofauna. Second, we provide an overview of several advanced methods that are being used for state-of-the-art research on stream meiofauna, including (1) the use of meiofauna in laboratory experiments assessing the effects of food quality and toxicants on surrogate measures of fitness; (2) how trophic relationships are investigated; and (3) how the movement and colonization dynamics of meiofauna are being studied. At the end of the chapter, we provide a list of the materials and supplies that are required for the basic methods; the advanced methods should only be undertaken after consulting the key references we provide.

II. GENERAL DESIGN

A. Site Selection

Studies of meiofauna may be either qualitative or quantitative, depending on the research goals. When selecting a research site, the investigator may wish to first just scoop up

fresh stream substrata and observe the material live. For quantitative ecological work, samples are easily collected and preserved using well defined techniques. For identification of major taxa, the researcher can use general references such as Higgins and Thiel (1988), Giere (1993), Smith (2001), or Thorp and Covich (2001). If more detailed (i.e., species-level) identifications are needed, the bibliographies of these sources should be consulted.

The new investigator may want to work in a relatively pristine stream and at a site with good water flow and medium to coarse substrata to observe the greatest meiofauna diversity. Finer substrata (e.g., mud) do harbor large numbers of animals, but samples must be collected only in the top layer of sediment and care must be taken not to "poison" the sample with deeper, anoxic mud prior to preservation. Additionally, in the warm, summer months animals left unpreserved will decay within hours.

B. Sampling

Qualitative Collection of Live Animals for the Classroom and for Experimentation

Examination of live fauna is quite instructive and a variety of data may be collected on locomotion, feeding, and sexual behavior. Additionally, for many taxa, species-level identifications cannot easily be made on preserved material (e.g., bdelloid rotifers, turbellarians, gastrotrichs).

Aliquots of substratum should be collected from the field by scooping sediment, leaves, moss, and rocks directly into buckets. The meiofauna can be concentrated by adding water to the bucket, swirling the sediment into the water to suspend the meiofauna, and then pouring the water through a sieve. The composition and abundance of the fauna collected depend strongly on the mesh size of the sieve (Hummon 1981). A 125-μm sieve[1] is fine for laboratory exercises; 40-μm sieves are more appropriate for research settings although this mesh size still may result in the loss of a many of the smaller meiofauna (Hummon 1981).

The animals retained on the sieve are then rinsed, using a wash bottle, into a second bucket with several liters of fresh stream water. The swirl-and-decant process should be repeated five or six times to ensure that an adequate sample of the fauna has been extracted from the sediment. This swirl-decant process can be performed in the field or in the laboratory as long as fresh stream water is used. If animals are to be examined later, it is imperative that the buckets of fresh material be kept cool. We routinely keep fauna alive for several days if we store the buckets in a cool environmental chamber or a refrigerator (e.g., 5°C).

Other methods for collecting live animals also exist. (1) Bou-Rouch pumps[2] are useful in streambeds that are difficult to penetrate (Figure 19.2). The pump stand has a perforated lower hollow column into which water and associated fauna can seep and from

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[1] Sieves are most economically made from Nitex® mesh and large plastic jars. Cut off the end of the jar and a large circle out of the jar lid. Secure the mesh under the jar lid by screwing the lid on tightly over the mesh.
[2] A "Bou-Rouch" pump can be made for about $100 US, using materials available at any plumbing store. Buy a wellpoint, punch through the screening of all openings in the lowest 15 cm of the pipe, and using bathtub caulking, seal up the rest of the openings (i.e., those above 15 cm). A hand pump that pulls a large volume per stroke (e.g., a "pitcher pump" in plumbing store jargon) works quite well. Occasionally, the caulk will have to be replaced, but this apparatus is durable, adaptable, and inexpensive.

FIGURE 19.2 Bou-Rouch pump for qualitatively sampling meiofauna from streambeds that are hard to penetrate with corers. The pump stand has a perforated lower hollow column into which water and associated fauna can seep and from which water is then pumped up and sieved. (Photo credit: Simon Rundle.)

which water is then pumped up and sieved (Bou 1974). (2) A turkey-baster or similar suction device is particularly useful for the qualitative sampling of the upper layers of muds and silts that can be sucked up and placed directly into a bucket. (3) Temperature gradients have been used to concentrate animals as many meiofauna move away from cold (freezing) surfaces (Higgins and Thiel 1988). (4) Bubbling of air into a bucket with fresh sediment causes many animals to float on the water surface; these animals may then be collected by skimming or by using blotting paper with subsequent rinsing. These and additional techniques are discussed by Higgins and Thiel (1988).

Quantitative Collection of Samples for Preservation

A vast array of sampling devices has been designed for streambeds, only a few of which we will mention here. If the substratum can be penetrated easily, the best and simplest sampling device is a corer made of PVC or clear acrylic pipe. If sampling is to

be relatively shallow, then a corer made out of a cut-off 30-mL syringe works quite well. The investigator should stand downstream of the sampling site and the corer should be inserted into the sediment in an area that has not been disturbed. If the sediment is fine enough (muds), a cork can be placed in the top of the corer and then the corer can be removed from the streambed without losing the sample. In all other types of substratum, the investigator will need to push his/her hand down under the core bottom before pulling the corer out of the streambed. Samples collected in gravel or sand substrata should be collected to a depth of at least 10 cm into the bed; in muds and silts, the coring can be shallower ($\approx$1 cm) because generally the depth of oxygen penetration is less.

For sampling deeper in sandy beds, a standpipe corer (Williams and Hynes 1974) works well. This corer allows one to collect intact samples from discrete depths in the streambed. For streambeds that simply cannot be cored, a Bou-Rouch pump (see above) may be used and has the advantage that it may be left in place between sampling dates. The disadvantage of pump sampling is that animals are often damaged and it is difficult to quantify samples; one can report numbers of animals per volume of water pumped but it is almost impossible to know from what area of the streambed this water originated. Another alternative is to use *in situ* freeze-coring devices. Bretschko (1990) has used this technique quite effectively and has minimized faunal avoidance of the sampler by electroshocking the area prior to sample collection, which stuns the animals so they don't migrate from the freezing surface.

Once samples have been retrieved from the streambed, they should be transferred to a sample container and several mL of 6% $MgCl_2$ anesthetic (73.2 g/L) added. The sample should be stirred and left to sit for ca. 5 minutes after which it should be rinsed through a sieve using fresh stream water. The contents of the sieve should then be rinsed back into the sample container using de-ionized water and a wash bottle. Several mL of 10% buffered rose bengal-formalin solution should then be added to the sample. Rose bengal (1 g/L stain in 10% formalin) is a protein stain that greatly facilitates microscopic sorting (but will kill live animals). Some animals will stain in 15 min but many require 48 hr for optimal staining.

III. SPECIFIC METHODS

A. Basic Method 1: Observing Live Meiofauna and Their Adaptations

It is useful for those new to the field of meiofauna biology to familiarize themselves with the dominant members of the meiofauna, their morphologies, locomotion, mode of reproduction, and feeding.

1. Use sand, mud, leaf, or algal material collected from the field and kept cool in buckets. To observe the live fauna, remove the bucket from the environmental chamber, suspend the settled substratum into the water, and pour an aliquot through a sieve. An aliquot of this sample can be transferred to a Petri dish for direct observation using a stereomicroscope. As the sample begins to reach room temperature, the animals will become more active and are easily observed. "Cool," fiber-optic light is preferred, but "warm," transmitted or reflected light is acceptable.
2. Examine the contents under a dissecting microscope and identify as many animals as you can to major taxonomic levels. If you do not have many animals in your sample, you can concentrate a larger volume from the bucket by swirling and decanting through a "live samples only" sieve (one that was not used for formalin

samples). While identification of meiofauna to genera or species is no easy task, you should be able to recognize the major groups using the stereomicroscope: bdelloid and monogonont rotifers, chironomid larvae, cyclopoid and harpacticoid copepods (adults and nauplii), oligochaetes, turbellarians, and nematodes. Depending on the material, you may be able to find gastrotrichs, tardigrades, cladocerans, and various insect larvae. A copy of Smith (2001), Thorp and Covich (2001), or other invertebrate text will facilitate this exercise.

3. Note the general differences in taxa, body shapes, and body sizes among different types of habitats. Animals from sand will probably contain small, slender representatives of each taxonomic group and may have more diverse (but smaller) fauna than those in mud or litter sample. Many of these sand-dwelling animals exhibit other adaptations for an interstitial existence including adhesive organs, such as the "toes" of rotifers and gastrotrichs. You should be able to find at least one of these structures by mounting an animal on a glass slide and observing it under a higher power on a compound microscope (see following). Adhesive organs are believed to reduce the chance of displacement from the highly mobile sandy substratum.

4. Copepod movement is very interesting to observe. Isolate copepods from different habitat types — for example, some from sand, some from mud, and some that you collected from litter samples. Note the difference in appendages (this may require high magnification). The sand-dwelling (interstitial) copepods, especially the harpacticoids, may have smaller legs than the copepods from the other substrata. The legs of these interstitial animals may closely adhere to the narrow or bullet-shaped body. Compaction of the legs close to a fusiform body makes it easier for the animal to "glide" among the interstices of sand. The mud-dwelling copepods will generally be larger than the interstitial forms and will have more robust bodies and stout appendages that are used to help the animal push through mud while it burrows. The leaf or algal-dwelling copepod will have a large, often somewhat flattened cephalothorax and appendages (especially the first leg), because it spends its life clinging and swimming among structures.

5. Isolate a few mud-dwelling worms in an area of the Petri dish to study their locomotion. Note that they are poor swimmers compared to the copepods. Nematodes have only longitudinal muscles and thus they make jerky, side-to-side movements; they move forward by pushing off a substratum. Oligochaetes are more adept burrowers than nematodes due to their well developed longitudinal and circular muscles. Rotifers have complex musculature and loop along by pushing and pulling, often using their toes or anterior end for adhering to a sand grain.

6. Depending on the time of year and how gentle you have been with your sample, you may be able to find individuals in various states of reproduction. Copepods are often found *in copula* within samples; the male grasps the female's urosome with his first antennae and will eventually pass a sperm sac to her (Figure 19.3). Eggs (usually 2–10) may be seen attached ventrally to her abdomen. Cladoceran eggs may be visible through the body wall. Aquatic nematodes may bear live young or lay eggs. Examine specimens under the compound microscope. You should be able to distinguish males from females by the presence or absence (respectively) of copulatory spicules that are used in sperm transfer. Sexual reproduction is rarely seen in rotifers; however, you may see eggs developing through the body wall. Some rotifers have separate sexes; however, most reproduction is parthenogenic. Freshwater meiobenthic oligochaetes may reproduce asexually or sexually, so you
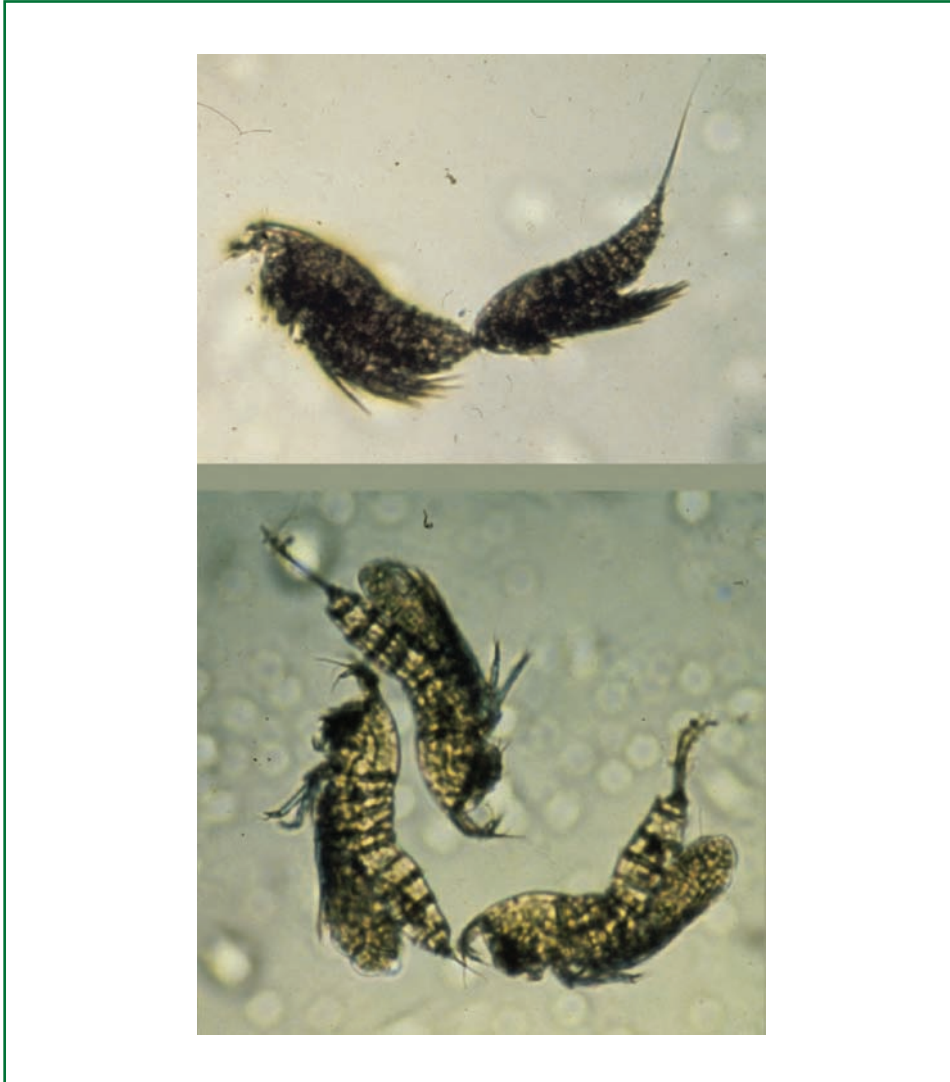
FIGURE 19.3 The life cycle of meiobenthic copepods involves direct development within aquatic sediments. Males pass a sperm sac to females while grasping them with their antennae (top photo). Females may fertilize multiple clutches with a single spermatophore; each clutch of eggs is held as a package on the underside of the female's abdomen (bottom photo) and young nauplii hatch out. (Photo credits: Bruce Coull.)

may find individuals in various states of budding, or see eggs (cocoons) attached to substrata in your sample. The immature chironomids you see are, of course, only one stage in the animal's life cycle. These larvae emerge at particular times of the year, and the adult lives a brief aerial existence before it flies back to the stream to lay eggs.

7. Meiofauna feed on bacteria, diatoms, other meiofauna, and protozoa. It is difficult to observe them feeding directly (unless you have live turbellarians), but you may find evidence of past feeding (Kennedy 1994). Most meiofauna are transparent

enough to see their gut contents. Be sure to mount some chironomids on glass slides and examine the guts for traces of diatoms or other animals. Often whole rotifers can be seen in their guts. In the digestive tract of rotifers, diatoms (or even other rotifers!) may be easily seen.

8. If higher levels of magnification are desired for further examination, animals should be transferred to a drop of water on a glass slide, a small piece of hair or wax inserted as a spacer, and a cover slip placed on top. Anesthetics (e.g., $MgCl_2 = 73.2 \, g/L$) may be added to the Petri dish sample to slow animals. This application will facilitate their transfer to slides using pipettes or Irwin loops. The latter are small, wire inoculating loops used in bacteriological work. Inexpensive, small-bore pipettes ("meiofaunal-sized") may be made by drawing out Pasteur pipettes (i.e., the pipette is heated and the tip lengthened to reduce the internal diameter). Once animals are on a slide, neosynephrine (available at any drugstore) or Protoslo® may be used as a narcotic. It can be bled under a cover slip by putting a drop along one side of the cover slip and letting it work its way under the slip. Alternatively, most animals can be slowed down by simply placing a tissue at the edge of the cover slip and drawing out just enough of the mounting water to squeeze the animal a bit.

B. Basic Method 2: Extraction and Identification of Preserved Meiofauna

1. To facilitate microscopic identification, animals may be extracted from preserved sediment using a variety of techniques. If the substratum is coarse (sand, gravel, cobbles), the best technique is a simple swirl-and-decant procedure. Pour the contents of the preserved sample onto the appropriately sized sieve and rinse with tap water to remove the formalin. Transfer the sample from the sieve into a 1000-mL Erlenmeyer flask. Add ca. 200 mL of tap water and vigorously swirl the sample to suspend fine particles and animals into the water. Quickly pour the supernatant through the sieve. Repeat this procedure five or six times and most of the animals will be extracted, assuming you properly relaxed the animals (see anesthetics above) prior to preservation. If this technique is being used for a quantitative study, the method must be "calibrated" for each sediment type. Using a dissecting microscope, the sorter should examine the sediment remaining in the flask after six decantations to be certain most of the animals were removed.

2. For fine sediments, either the entire sample must be microscopically examined (fortunately, much less sediment is collected when sampling muds) or a more complicated extraction procedure used. Many workers employ a density gradient technique. Here, the preserved sample is rinsed into a 100-mL Nalgene centrifuge tube. The tube is filled with water and the sample centrifuged at high speed for several minutes. To remove formalin and excess water, the supernatant is poured through a sieve, with the sample "pellet" left in the tube. Occasionally a few animals will be retained on the sieve, so the contents of the sieve should be rinsed into a jar to be used for your extracted fauna. About 50 mL of a colloidal silica solution (Ludox-TM® distributed by DuPont and described in DeJonge and Bouwman 1971) is added to the sample pellet. The sample should be stirred thoroughly for several minutes to be certain that the entire pellet and associated animals are suspended. The sample is then centrifuged at a lower speed (500–1500 rpm) for 3–5 min. Pour the supernatant into the sieve and, using a wash bottle, carefully rinse the sides of

the tube (but not the sediment pellet) into the sieve. Most of the animals will be floating in the silica supernatant. As with the decantation procedure, this process requires calibration for each sediment type. The centrifuge speed and time greatly affect the separation of animals from sediments. This technique is described in more detail and compared to other procedures in Pfannukuche and Thiel (1988).

3. Once the animals have been extracted from the sediment, a small amount of animal material (see Fleeger *et al.* 1988 for a discussion of subsampling) should be placed in a gridded Petri dish. Small rectangular dishes (ca. 10 cm × 5 cm) with shallow sides (ca. 1.5 cm depth) constructed of thin Plexiglas® work well and can be made inexpensively. The bottom can be "gridded" by simply scratching the surface of the Plexiglas; the size of the grids should correspond to the stereoscopic field of vision. The sample, which will contain some sediment, can then be scanned (one grid square at a time) at 12–18X to locate meiofauna. Higher magnification (up to 50X) on the stereoscope is desirable as many of the rotifers are small and this higher magnification is necessary to identify them. It is wise to periodically check the efficiency of this process by having one or more individual investigators count a single sample several times. "Removal" statistics adapted from fisheries science can be used to calculate sorting efficiencies on such repeatedly counted samples (e.g., Zippin 1958).

4. For more exact identification, the animals should be transferred to a drop of glycerol on a glass slide, and viewed with a compound microscope. If a cover slip crushes the animals, place several very small pieces of hair under the cover slip. Hulings and Gray (1971) provide a more lengthy discussion of mounting techniques. Semipermanent slides can easily be made by mounting specimens directly from water, alcohol, or formaldehyde into the CMC series of mounting media (available from the Masters Chemical Company, Inc., 520 Bonnie Lake, Elk Grove, IL 60007), which clears the specimens. Ring the cover slip with clear nail polish after a day or two, and the slide can last for years.

Identification to major taxonomic groups is not difficult (Table 19.1). A simple key for the identification of major groups of stream meiofauna is available free on the Internet at http://www.ecostudies.org/meiofauna_key.html. Most meiofauna can even be readily identified to the level of family using keys in Smith (2001) and Thorp and Covich (2001). Identification to genus or species may be difficult, and often requires experience, special techniques, and consultation of the primary taxonomic literature. Smith (2001), Thorp and Covich (2001), and references cited therein provide an introduction to these specialized taxonomic techniques and references.

C. Advanced Method 1: Determining the Effects of Food Quality and Toxicants Using Meiofauna

Many meiofaunal taxa are highly amenable to laboratory culture and experimentation. Their small size and relatively short generation times mean that they can be maintained in high numbers on a small scale and that measures that relate to fitness can be obtained rapidly. Here we describe a laboratory protocol for studying the effects of food quality and environmental toxicants on freshwater harpacticoid copepods.

TABLE 19.1	Sample Data Sheet for Identification and Enumeration of Meiofauna.

Sample site: _____   Date: _____   Sorter: ____

Taxa	Notes	Sample 1	2	3	4	5	Mean	%
Chironomids								
Cladocerans								
Copepods								
Gastrotrichs								
Nematodes								
Oligochaetes								
Ostracods								
Rotifers								
Tardigrades								
Turbellarians								
Total Meiofauna								

Establishing a Laboratory Culture

1. Cultures of animals should be established two to three months before experimental trials and the opportunity taken during their establishment to practice handling, observing, and identifying different life stages (see following).
2. A sample of meiofauna should be collected as outlined above, observed live in the laboratory (Basic Exercise 1) and harpacticoid copepods (females with eggs or mating pairs; Figure 19.3) extracted using a fine pipette. Animals (or pairs of animals) should be placed into separate small containers (e.g., 1 cm-diam Cellwells® that come in blocks of 15) sorting them by size and general morphology under a stereomicroscope. Chances are good that there will be several species at the site sampled; sorting animals in this way should increase the likelihood that only one species is cultured.
3. Cultures can be initiated by placing individual females or mating pairs in larger Petri dishes (10 mm diam.) containing growth medium (e.g., ASTM 1980) and leaf fragments (conditioned in growth medium — see following). Ideally, cultures and trials should be maintained at a constant temperature (15–20°C) with a day-night light regime and water changed every two to three days. Established cultures (i.e., after four to five weeks) will need to be split if densities get too high.

Measuring Development and Reproduction

1. Development times and reproductive success can be used as surrogate measures of the influence of food quality and other environmental parameters on copepod

fitness. At 20°C, development times for many harpacticoids are 15–20 d; expect development times of ca. 10 d longer for trials at 15°C.

2. Developmental trials should be started using nauplii obtained from ovigerous females isolated from cultures. Nauplii should be maintained individually in 1-cm Cellwells® containing 2 ml of medium and a 4 mm-diam conditioned leaf disc (see following).

3. Copepod survival and the presence of moulted exuviae should be recorded daily (Table 19.2) and used to calculate the duration of the combined nauliar stages (Dn), individual (C1, C2, C3, C4, C5) and combined copepodid stages (Dc) and total development time (i.e., N1-A, where N1 = the first naupliar stage and A = adult) (Brown *et al.* 2003).

4. The Dc/Dn ratio can be used to investigate an index of food availability during development; development times are predicted to decrease more with increased food quality/quantity for copepodids than for nauplii (Brown *et al.* 2003, Hart 1990, 1998). The duration of individual copepodid stages allows one to test for equiproportional development (see following).

5. Reproduction trials should be conducted using newly mated pairs from cultures—that is, mating pairs where the female has no egg sac. Pairs should be placed in Cellwells® and be maintained as for nauplii but with a larger (10 mm diam) leaf disc.

TABLE 19.2	Measuring Development Rate and Reproduction for Meiobenthic Copepods. A check mark should be placed in the appropriate column of the table if that stage is present. Each well starts with one nauplius (N). While there are six naupliar stages, they are all listed as a single category because of their similarities, while the transition to the copepodite (C1) stage is dramatic and obvious. Transitions to the remaining copepodite stages are best determined by looking for exuviae. Each time one is found, it should be removed from the well. $Dn = \sum$ of days in C1 (e.g., Date 5 − Date 3 below); $Cn = \sum$ of days in C1-C5 (see text).

Well #1

Date	N	C1	C2	C3	C4	C5
Date 1	✓					
Date 2	✓					
Date 3		✓				
Date 4		✓				
Date 5			✓			
.						
.						
.						
Date i						

6. Daily observations of the presence of egg sacs and nauplii can be made to calculate the embryonic development time (i.e., the time from egg sac release to hatching) and the number of broods per female.
7. Transfer pairs to a new Cellwell® (with new media and leaf disc) every seven days and count remaining nauplii (those from separate broods can be distinguished by size) and unhatched eggs allowing you to calculate embryonic development times, numbers of broods per female, hatching success, and total offspring production.

Experimental Treatments

1. Copepod development and reproduction can be used to assess food quality or the impacts of a toxicant (Chandler and Green 1996, Chandler and Scott 1991, DiPinto *et al.* 1993). Cultured individuals are simply placed in dishes with the appropriate treatment and followed over time. For example, the sublethal effects of toxicants could be investigated by using treatments containing varying levels of pollutants such as trace metals (Burton *et al.* 2002) or pesticides (Brown *et al.* 2003).
2. Of particular use for assessing the effects of toxicants is the deviation from Equiproportional Development (ED) where the proportion of total development time copepods spend in each moult stage is constant; ED is predicted to be disrupted when toxicants affect developmental processes such as moulting (Hart 1998, Brown *et al.* 2003).
3. As a second example, the value of stream leaf litter as food can be assessed by providing animals with leaf discs conditioned for different periods of time or with different types of leaf species (Brown *et al.* 2003). Leaves could be collected from the catchment of the stream from which you collected animals, then dried and conditioned in the experimental medium (Swan and Palmer 2004).
4. Ideally, the microbial assemblages in the leaf biofilms (the copepods' "food") should be quantified (densities of bacteria, algae, and fungi) before and after trials (see Brown *et al.* 2003). Differences in developmental and reproduction parameters and in microbial components between treatments (Palmer *et al.* 2000) can be assessed using ANOVA or a similar non-parametric statistical test. As there may be substantial variability in these measures between individuals, at least 15 replicate animals should be used per treatment.

D. Advanced Method 2: Determining Trophic Relationships

1. The meiofauna are enormously diversified in their feeding habits, and include suspension feeders, bulk deposit feeders, biofilm scrapers, selective grazers of benthic algae, grazers of vascular plants, highly specialized predators, generalist predators, and parasites (Rundle *et al.* 2002). There are several ways, simple or sophisticated, to learn about the trophic relationships of meiofauna. The simplest is direct examination of the animal and its gut contents. Most meiofauna are so transparent that some of their mouthparts and gut contents can be observed directly, either on living animals or on dead animals that have been cleared and mounted on microscope slides. If living animals are to be observed, you may have to slow them down by squeezing them under a cover slip or narcotizing them (see basic methods above). Killed animals may be mounted and cleared in CMC mounting media, then observed under a compund microscope. Look in the animal's gut for detritus particles, diatom frustules, rotifer loricas or mouthparts,

crustacean carapaces, legs, and claws, oligochaete setae, insect head-capsules, and other structures that resist digestion. It may be helpful to examine a sample of sediment and other animals from the same site to aid in identifying the bits and pieces of food you find in the animal's gut. Gut contents are not a perfect reflection of diet because some items in the gut may be indigestible, highly digestible food may disappear before you see it, and much material in the gut (especially detritus) may be unidentifiable. Nevertheless, cautious analyses of gut contents have been used in quantitative analyses of meiofaunal food webs (e.g., Strayer and Likens 1986, Schmid and Schmid-Araya 2002).

2. You may also get a clue to the animal's diet by looking at its mouthparts. For example, tardigrades and dorylaimoid nematodes have hollow spears and muscular pharynxes for piercing their prey and pumping out the cellular contents. Rotifer mouthparts are highly diversified, depending on diet (Pourriot 1977). Researchers have even made formal schemes for relating mouth structure to diet in nematodes (e.g., Jensen 1987).

3. Ecologists commonly use biochemical and isotopic tracers to follow trophic relationships in food webs (see Chapter 27). These techniques have been used only rarely on meiofauna because of technical difficulties in analyzing small samples. Nevertheless, marine ecologists have begun to use both tracer additions and natural abundance of stable isotopes (^{13}C and ^{15}N) to elucidate the diet of meiofauna (Middelburg *et al.* 2000, Carman and Fry 2002). Fatty acids may also be useful tracers of meiofaunal diets (Coull 1999). These techniques are likely to become more important in analyzing the trophic relationships of meiofauna as technology improves.

E. Advanced Method 3: Determining What Influences Movement and Colonization Dynamics

1. Meiofauna are ideal animals for studying recolonization processes in streams because they are small, easy to enumerate, and recover rapidly from disturbances (Coull and Palmer 1984). They may disperse through the water column via the drift or move laterally through the streambed. Movement through the sediments is much slower and accounts for far less dispersal than water column movement (Palmer *et al.* 1992). Conversely, because water flow enhances the entry of meiofauna into the water column, lotic meiofauna account for a large, yet often ignored, proportion of stream drift (Palmer 1992, Schram *et al.* 1990). Meiofauna are largely unable to avoid transport once they move into flowing water and thus "passive drift" (dislodgment due to water flow) commonly leads to redistribution of meiofauna on both small and large scales (Robertson *et al.* 1995, Palmer *et al.* 1996). Although travel distances of individual meiofauna are poorly known, distances of 10's of meters are likely when flows are moderate and distances may exceed hundreds of meters at high flows (Palmer *et al.* 1996). After floods, drift is a major source of meiofauna recolonization (Marmonier and Creuze de Chatelliers 1991, Robertson *et al.* 1995, Palmer *et al.* 1996, Robertson 2002). Despite their propensity to drift, there is now ample evidence that they can exert some control over final benthic site "selection" since some taxa are known to exhibit significantly higher abundances in certain habitat types and habitat spatial arrangements despite being repeatedly dispersed by high flows (Silver *et al.* 2000, Silver *et al.* 2004).

2. Meiofauna are also known to move into the water and even disperse through the sediments in response to predators and predatory cues (Shofner 1999, Silver *et al.*

2002). Experimental work to examine their movement response to predators and predatory cues has involved complex field experiments in which defaunated sediment is buried in areas surrounding predator enclosures and meiofaunal movements into that defaunated sediment can only occur from within the cage sediment (see Palmer and Strayer 1996 for basic methods). The defaunated sediment is later sampled to enumerate the meiofauna that have colonized it (Shofner 1999). Laboratory experiments have also been performed in which meiofauna movements at the sediment-water interface and up into the water have been quantified and shown to be significantly influenced by the presence of predator cues (fish "juice"; Silver *et al.* 2002). Other researchers have used flumes to document very small vertical migrations of some meiofauna taxa deeper into stream sediments as flow is increased (Coull *et al.* 1989, Palmer *et al.* 1992). To date, we know of no successful attempts to label meiofauna (e.g., fluorescently) and track their dispersal in the field; recovery rates of "tagged" and released meiofauna are simply too low to yield statistically reliable results (Palmer unpublished data).

IV. QUESTIONS

1. How might patterns in the species composition, abundance, and spatial distribution of meiofauna differ in fast-flowing cobble streams versus low-gradient, sandy streams with large amounts of woody debris? Consider not only differences in the taxa that might be expected in each stream but also in their dispersal abilities and feeding habits.
2. Given their vast difference in modes of locomotion and reproduction, you might expect meiofauna to differ greatly in their dispersal abilities. Which taxonomic group(s) would you expect to have the greatest dispersal potential? The lowest potential? Why?
3. Some of the animals you observed reproduce asexually while others have separate sexes and internal fertilization. What are the advantages of each mode of reproduction for these fauna? If a stream is prone to unpredictable, severe disturbances (e.g., floods, droughts, etc.), which reproductive mode might prevail? Why?
4. A small stream is known to be receiving pollution from a copper mine. How could you use a combination of field sampling and laboratory trials to assess if this pollution was likely to be impacting copepod populations at this site?
5. Are meiofauna just little macrofauna, or are they biologically and ecologically distinctive? Think about how body size might affect the diets, degree of feeding specialization, response to physical disturbance, and dispersal of stream-dwelling animals.
6. The margins and floodplains of streams are dry for part of the year and underwater for the rest of the year. What kinds of meiofauna might live in this zone? What biological and ecological traits would you expect these animals to possess?

V. MATERIALS AND SUPPLIES

Field Materials

> 6% $MgCl_2$ solution (73.2 g/L)
> 10% buffered formalin (to buffer, saturate with sodium borate)

125-μm or smaller mesh sieve
Buckets (that have not been exposed to formalin)
Corer (ca. 2 cm diam, 10 cm long; can be made from 30-mL syringe)
Rose bengal stain (add 1 g of rose bengal powder per liter of 10% formalin)
Sample jars
Trowel or piece of plastic cut from a milk container for scooping up sediment
Wash bottles

Laboratory Materials

125-μm or smaller mesh sieve
1000-mL Erlenmeyer flasks
Micropipettes
Irwin loops or small-bore Pasteur pipettes
Microscope slides and cover slips
Plastic petri dishes or other sorting trays
Protoslo®, $MgCl_2$, or other anaesthetizing agent
CMC mounting media
Stereomicroscope

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Macroinvertebrates

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I. INTRODUCTION

Macroinvertebrates are virtually ubiquitous in the streams and rivers of the world. Only the most harsh, temporary, or grossly polluted lotic environments do not contain some representatives of this diverse and ecologically important group of organisms. By convention, the term "macro" refers to invertebrate fauna retained by a 500-μm mesh net or sieve. However, the early life stages of many macroinvertebrates pass through mesh openings of this size. Yet, these early stages are important to understanding species-specific life histories, trophic relations, secondary production, and a multitude of other ecological relationships. Thus, a general trend among stream ecologists is to use collecting methods employing finer-meshed collecting nets (e.g., 125 to 250 μm). Although organisms passing through a 500-μm net, but retained by a 40-μm net, are considered meiofauna (see Chapter 19), stream ecologists studying macroinvertebrates, especially aquatic insects, have tended to include the early life stages of organisms considered "macro" in their mature stages.

Streams and rivers contain a remarkable diversity of macroinvertebrates. In many lotic environments, the macroinvertebrate community consists of several hundred species from numerous phyla (e.g., Morse *et al.* 1980, Benke *et al.* 1984, Roy *et al.* 2003, Hose *et al.* 2004) including arthropods (insects, mites, scuds, and crayfish), mollusks (snails, limpets, mussels, and clams), annelids (segmented worms and leeches), nematodes (roundworms), and turbellarians (flatworms). Most stream macroinvertebrate species are benthic; that is, they are associated with surfaces of the channel bottom (e.g., bedrock, cobble, finer sediments) or other stable surfaces (e.g., fallen trees, snags, roots, and submerged or emergent aquatic vegetation) rather than being routinely free-swimming. However, many

of the swimming insects found in ponds (e.g., the water boatmen) or even in quiescent stream pools (e.g., diving beetles) are the most conspicuous insects we see.

In large, alluvial gravel-bed streams and rivers the hyporheic zone (see Chapters 6, 33) often contains zones of preferential flow characterized by large interstitial space between sorted cobble layers. Numerous species of amphibitic stoneflies spend most of their nymphal life histories in the hyporheic zone of stream and river floodplains, returning to the main channel to emerge as adults and reproduce (Stanford *et al.* 1994, Stanford 1998).

Partly because of their importance within the stream community as a fundamental link in the food web between organic matter resources (e.g., leaf litter, algae, detritus) and fishes, and partly because of their diversity and ubiquity, the study of macroinvertebrates has been (Hynes 1970, Cummins 1974, Allan 1995), and will continue to be, a central part of stream ecology (Hauer *et al.* 2000, Boyero and Bailey 2001, Lamouroux *et al.* 2004). Earlier chapters within this book have focused on the multitude of interactive physical, chemical, and biological variables that constitute the stream ecosystem. For example, geology, climate, and other landscape features directly affect hydrologic patterns, and the movement and storage of inorganic and organic materials. Nutrients and the downstream transport of solutes are affected by channel and substratum complexity, the interactions of ground and surface waters, and by the stream biota. Interactions between the stream channel, hyporheic zone, and riparian floodplains likewise are important features in the structure and function of the entire stream corridor (Ward 1997, Stanford *et al.* 2005). These and many other factors affect the microhabitat structure of the stream and therefore the distribution and abundance of stream macroinvertebrates.

A. Phylogeny and Adaptations

The origin of stream macroinvertebrates includes groups that are terrestrially derived (e.g., the insects) and groups that are marine in origin (e.g., mollusks and crustaceans). Of the various taxonomic groups that comprise the stream macroinvertebrate community, no group has been studied more than the aquatic insects. Not only are the aquatic insects extremely diverse, both taxonomically and functionally, but they also are frequently the most abundant large organisms collected in stream benthic samples. For example, 13 orders of aquatic insects occur in North America (Merritt and Cummins 1996), although only five of these are composed strictly of aquatic species (i.e., species that have at least one life-history stage that is obligatorily aquatic): the dragonflies and damselflies (Odonata), the stoneflies (Plecoptera), the mayflies (Ephemeroptera), the caddisflies (Trichoptera), and the hellgrammites (Megaloptera). Although the remaining eight orders have primarily terrestrial inhabitants, several of these orders exhibit high species richness (often thousands of species) in aquatic habitats. For example, the beetles (Coleoptera) and true flies (Diptera) each contain more aquatic species than are found among any of the completely aquatic orders.

The phylogeny of aquatic insects is part of what makes these organisms so interesting. No single line of aquatic insects evolved, but rather insects invaded the freshwater environment many different times and in many different ways (Resh and Solem 1996). As a result, problems of living in the stream environment, such as how to obtain oxygen or remain in a fixed position, have been solved repeatedly. Moreover, the mechanisms developed to overcome these obstacles involve a variety of different approaches and morphological adaptations. For example, some lotic species have developed structures to obtain oxygen from the atmosphere (analogous to snorkeling), others use the temporary storage of an air bubble (analogous to SCUBA diving), a few species use respiratory pigments (analogous to vertebrate hemoglobin), and many species have developed tracheal gills for obtaining oxygen dissolved in the water (Eriksen *et al.* 1996). Likewise, morphological adaptations

for existence in a running water environment include sclerotized projections along trailing edges of legs and body to form hydrofoils that press the organism onto the substratum, streamlining of body shape to offer reduced resistance while swimming, suckers and modified gills to attach to smooth surfaces, and leg and anal hooks to attach to a variety of surfaces, to name but a few (Resh and Solem 1996). The Trichoptera, Lepidoptera, and Diptera also use silk in a myriad of ways such as for attachment (e.g., free-living caddisflies and black flies), food gathering (e.g., net-spinning caddisflies), and shelter construction (e.g., midge larvae, moth larvae, and cased caddisflies).

Life history features that govern the reproduction and survival of lotic macroinvertebrates also show adaptations to specific characteristics of running water environments. Many stream environments are very dynamic (hydrologically, spatially, thermally, trophically, etc.), and macroinvertebrate life histories reflect this through tremendous diversity and adaptability (Butler 1984). For example, some species are specially adapted to ephemeral streams by having dormant egg stages that hatch as they are hydrated when flow resumes (Williams 1987). Also, closely related species that perform a similar trophic function may temporally separate growth and adult emergence within the same stream reach (Hauer and Stanford 1982a, 1986). Other life history adaptations can be seen in the seasonal timing of larval diapause (Gray and Fisher 1981) or pheromone release by adults for mate attraction (Resh *et al.* 1987). There is also considerable variation in the length of life cycles—some species may have several complete life cycles per year (multivoltine), two life cycles per year (bivoltine), one life cycle per year (univoltine), or may require two or three years to complete a life cycle (semivoltine). Specific life histories also may be very different across the geographic distribution of a species, where in one portion of its range a species may be univoltine and in another portion (generally colder) it is semivoltine. For example, the limnephilid caddisfly *Dicosmoecus gilvipes* is univoltine in coastal streams of California and Oregon (Lamberti *et al.* 1987) but semivoltine in mountain streams of Montana (Hauer and Stanford 1982b).

Behavioral adaptations are evident in aquatic insects as well, and these include regulatory behaviors to increase the control that an individual exerts over its own metabolic status, foraging behavior that involves the gathering and processing of food resources, or reproductive behavior that is responsible for the successful continuation of life into the next generation (Wiley and Kohler 1984). For example, *behavioral drift*, the intentional entry of benthic animals into the water column and their subsequent downstream transport, is a topic that has greatly interested stream ecologists for over three decades (Waters 1972, Müller 1974, Brittain and Eikeland 1988; see also Chapter 21) and may be essential to colonization processes, search for food, or predator avoidance.

Hydrologic processes, food resources, nutrient dynamics, riparian vegetation, and many other factors intimately affect the structure and function of stream ecosystems (Stanford *et al.* 2005). A fundamental characteristic of these factors is that they change along the longitudinal profile of the stream ecosystem (Vannote *et al.* 1980), and these factors may be affected by various anthropogenic influences (e.g., stream regulation; Stanford and Ward 2001). Macroinvertebrate species composition also changes between headwaters, middle reaches, and large rivers, in response to changes in the stream environment. For example, a stream reach flowing through a deciduous forest with a dense overhanging canopy may have a large number of macroinvertebrates that specialize in feeding on leaf litter, but that same stream upon entering a meadow (and thus having an open canopy) may be dominated by species that graze on periphyton. Within functionally similar groups (e.g., those that feed on similar food resources and use similar feeding mechanisms; see Chapter 25 and Merritt and Cummins 1996), species replacement along the river continuum is also very common. For example, among the net-spinning

caddisflies (Hydropsychidae) numerous species may occur within a large river basin and be distributed in a very predictable manner along the longitudinal stream profile (Hildrew and Eddington 1979, Hauer *et al.* 2000). Some species occur only in first- and second-order streams, other species replace the headwater species in third- through fifth-order middle reaches, and still other species will only occur in larger rivers (Hauer *et al.* 2000). Each species of hydropsychid spins a silk-thread catchnet that filters food particles from the flowing waters with different levels of efficiency (Edler and Georgian 2004). Yet, through selection of particular habitats, food resources, and temperature regimes these species exhibit very predictable landscape-scale distributions. This phenomenon is not restricted to the hydropsychid caddisflies, but rather occurs among the various species within trophic and phenologic groups (Hauer *et al.* 2000) (Figure 20.1).

People collecting stream macroinvertebrates for the first time are often amazed at both the complexity of the community and the wondrous variety of habitats in which they are found. Some species exist exclusively in very turbulent, high-velocity waters where they use sucker discs, hooks, or silk to remain attached to the substratum. Other species occur in pools where stream currents are slow and their specialized body structures permit them to move across the fine sediments that accumulate in pools and backwaters. Many species can be found in leaf packs where they are surrounded by the food they eat and still others bore under the bark and through the boles of large wood debris that has fallen into the stream or river (Merritt and Cummins 1996; see also Chapters 13, 25, and 30).

In this chapter, we describe several approaches to the study of stream macroinvertebrates. The purpose of these exercises is to expose stream researchers to various field and laboratory methods for the collection and study of macroinvertebrates. Over the past three to four decades, thousands of studies have focused on stream macroinvertebrates. Numerous detailed scholarly works dedicated to macroinvertebrate collection and analysis also exist, such as collecting and sampling (Merritt *et al.* 1996), sampling design (Resh 1979, Norris *et al.* 1992), and statistical analyses and study design (Elliott 1977, Norris 1995, Hawkins *et al.* 2000). The purpose of this chapter is neither to synthesize nor replace these detailed examinations but rather to introduce stream ecology students or researchers that have not worked previously with macroinvertebrates to this interesting and diverse group of organisms. Summaries of the biology of the different orders of aquatic insects are presented in Resh and Carde (2004).

Although the collecting methods we describe are most easily performed in wadeable, small to midsized streams, they can be adapted to larger rivers. While applying the various methods and exercises described following, note the tremendous variety of habitats and the different ways in which macroinvertebrates are adapted to use resources. The specific objectives of this chapter are to (1) familiarize students and researchers with a variety of techniques for sampling stream macroinvertebrates; (2) describe how to preserve and process samples for laboratory examination; (3) introduce the concepts of abundance and diversity of stream macroinvertebrates; (4) examine large-scale distribution patterns; and (5) investigate microhabitat utilization and movement. We give only examples of approaches to be taken.

II. GENERAL DESIGN

A. Field Sampling

Over the past several decades, many different types of sampling devices have been invented for the systematic collection of stream macroinvertebrates, yet only a few standard sampling devices are used for most studies. Stream macroinvertebrates generally can be
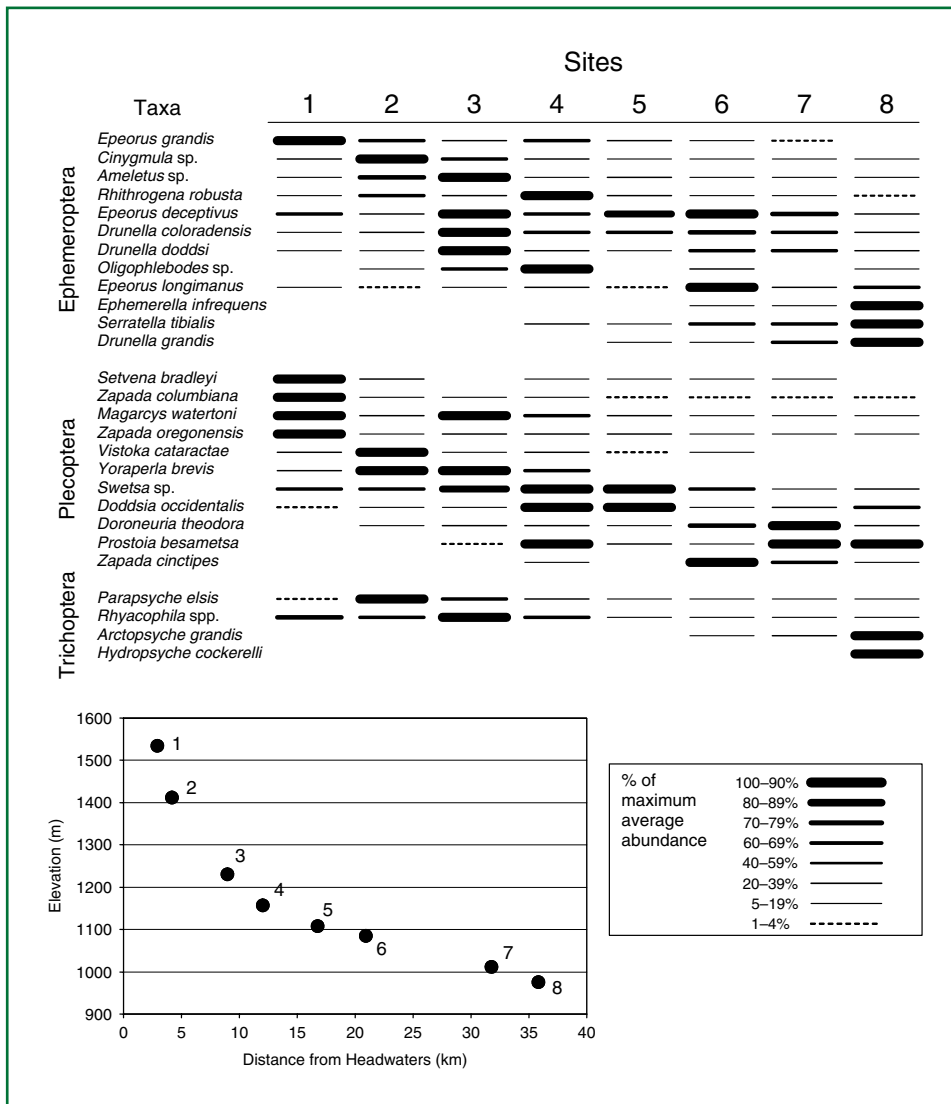
FIGURE 20.1 Frequency of macroinvertebrate fauna collected along the longitudinal and elevation stream gradient of McDonald Creek in Glacier National Park, Montana (modified from Hauer *et al.* 2000).

collected by disturbing bottom sediments (e.g., gravel, cobble) and catching organisms in a net held downstream. Most samplers are designed to delineate a certain area of stream bottom (e.g., $1\,ft^2$, $0.25\,m^2$, $0.5\,m^2$). Then, by disturbing the substratum materials the benthic macroinvertebrates are dislodged and captured as they are swept into the net by the current.[1] Merritt and Cummins (1996) provide an excellent review of various macroinvertebrate samplers used in streams and rivers.

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[1] A video (Resh *et al.* 1990) that demonstrates over 20 collecting techniques usable in a variety of stream habitats is available through the Office of Media Services, University of California, Berkeley or by contacting VHR at vresh@nature.berkeley.edu.

The Surber sampler (Surber 1937) and Hess sampler (Hess 1941) are two standard collecting devices for stream macroinvertebrates that have been used widely in stream ecology for over 60 years. Both samplers generally are small and limited to sampling stream depths <15 cm and in streams with small substrata (i.e., sand, gravel and very small cobble). Another standard collecting device for stream macroinvertebrates is the kicknet, so-named because of the kicking action done in front of the net. The simplest kicknet is easy to make; use two wooden dowels about 1.25 m long and 2–3 cm in diameter and attach a 1 m × 1 m square of 500-μm mesh Nitex® netting to each dowel (Figure 20.2). The net is held perpendicular to the flow and the substratum is disturbed in front of the net. The organisms are collected on the net surface after the net is removed from the stream. This collecting net has the major drawback of often rapidly filling with material that clogs the net and results in back welling. As the net is no longer filtering water the organisms are swept back out of the net, thus adding sampling error to the collection.

We have found that for large cobble to small boulder size substratum (10–30 cm), a modification of the kicknet takes advantage of the kicknet's size and allows for efficient sampling of substratum that is too large for Surber or Hess samplers and more effi- cient than a D-frame sampler. The Stanford-Hauer kicknet (Hauer and Stanford 1981) combines the size and principal of the kicknet with the filtering capacity of the Surber sampler and unlike the Surber can be used in deep (20–40 cm), swift (>50 cm per sec) riffles and runs. Operation of the Stanford-Hauer kicknet may be done with either one or two persons. In the two-person operation, one person opens the kicknet and lowers the net base to the substratum oriented perpendicular to the stream current (Figure 20.3A). The second person places a 0.5 m^2 frame made of 1/4- or 3/8-inch diameter steel rebar in front of the net. Then cobble from within the frame is disturbed one stone at a time, and carefully brushed and washed. The organisms from each stone are washed into the net by the current. The sample area is then vigorously disturbed by stepping into the framed area and kicking back and forth for about 30–60 sec. The Stanford-Hauer kicknet can also be operated by a single individual if the current is not too strong (Figure 20.3B).

Numerous sampling devices also have been designed for collecting macroinvertebrates from other stream substratum types. Coring or dredging devices have been used to sample soft sediments such as sand or mud and frequently are necessary for sampling in large rivers or wherever soft sediments are prevalent. The D-frame net may be used to sample gravel or cobble substrata, soft sediments, or woody snags (Figure 20.2).

After a sample is collected, the organisms are rinsed into the end of the net (or in the case of the Surber or Stanford-Hauer kicknet sampler illustrated in Figure 20.3, into a detachable, meshed cup). At streamside, the contents of the sample are poured through a fine-meshed net or sieve of 125 μm or less, depending on original mesh size of the sampler, to remove excess water from the sample. If the sample is large it may be washed into a 20-L plastic bucket prior to transfer into the sieve. Samples that are to be returned to the laboratory should be placed into a plastic jar or Ziploc® bag and preserved in a 70% ethanol solution. This may be best done by adding 95% ethanol to the sample and estimating the remaining water, body fluids, and other organic matter to dilute the ethanol concentration to 70%.[2] Ideally, samples should be sorted within 24–48 hr after collection to prevent specimens from deteriorating. In some cases, immediate live-sorting may be

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[2] Traditionally, samples were preserved with 5% formalin solution or Kahle's fluid (28% ethanol, 11% formalin, 2% glacial acetic acid, and 59% water). Although formalin and Kahle's are very effective preservatives, they are hazardous to use and difficult to dispose of after use. They should not be used for classroom purposes.
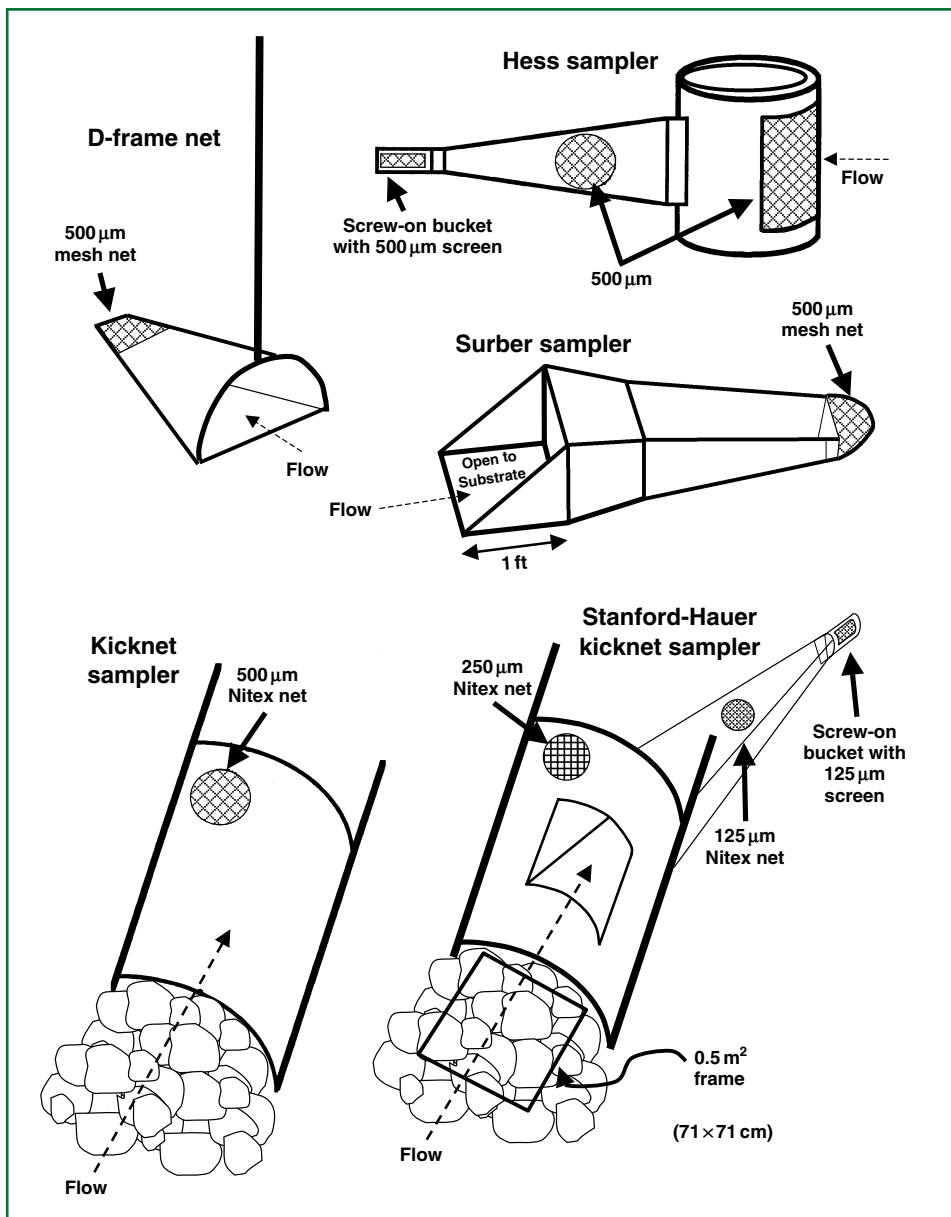
FIGURE 20.2 Illustrations of the most common sampling devices used to capture macroinvertebrates in streams with gravel and cobble bed material.

useful, assuming samples are kept cool. After identification, stream macroinvertebrates that are intended for long-term storage should be curated in a glass patent-lip vial with a neoprene stopper, clean 70% ethanol, and a proper label.

By using a Surber, Hess, or Stanford-Hauer kicknet sampler (or some other sampling device with a defined area), quantitative samples may be collected from a known area and in a standardized fashion to obtain sample replicates. From these replicates a sample mean

FIGURE 20.3 Field pictures of the Stanford-Hauer kicknet being used by (A) two persons and (B) by a single person. Note the extended net and bucket end of the kicknet sampler.

and variance can be calculated to estimate population size and variability. Quantitative sampling is necessary for most ecological investigations and involves a variety of decisions, including choice of sampling sites, depth of penetration of sampling into the substrata, frequency of sampling, and other decisions. Bias (i.e., the lack of congruence between

what is in a sample and what actually occurred in the sample area) can result from factors related to the characteristics of the sampler (e.g., backwashing of the sample from a clogged net), the organisms being sampled (e.g., tight attachment to substrata, movements to avoid being caught), and inconsistency among the users of the sampling devices (Resh 1979). The nonrandom distributions of most stream macroinvertebrate populations may require that large numbers of samples be collected in quantitative studies.

B. Laboratory Procedures

Sorting, which is done in the laboratory, involves the separation of the benthic macroinvertebrates from the substrata, organic matter, and other unwanted material found in the sample. Sorting can be time-consuming, but using sieves to separate out larger particles, dyes to stain macroinvertebrates (e.g., rose bengal; see also Chapter 21), adding sugar to change the specific gravity of the liquid and thus "float off" organisms, and subsampling of very large samples can all greatly reduce sorting time and effort. Large organisms can be easily seen and sorted without the aid of magnification; however, small species (e.g., microcaddisflies, midge larvae) and early instars of even the large species of macroinvertebrates require scrutiny under a good dissecting microscope.

After samples have been sorted, organisms must be identified and counted. The results are then analyzed by various procedures depending on the research questions and the accompanying experimental design. By following simple keys based on distinguishing morphological characteristics, it is relatively easy to identify macroinvertebrates to the family level. However, distinguishing macroinvertebrates at the generic or species level generally requires substantial training. What level of identification is required? The answer depends on the objective of the study (see discussion in Lenat and Resh 2001). We have included a simple flow-key to the more common stream macroinvertebrates (Appendix 20.1). Detailed keys to the families of North American freshwater invertebrates (Thorp and Covich 2001), and to the genera of North American aquatic insects (Merritt and Cummins 1996) and noninsects (Pennak 1989), are excellent starting points for more detailed identification of stream macroinvertebrates. McCafferty (1981) has excellent illustrations of aquatic insects to the family-level. Species-level keys are usually confined to a single genus (e.g., Szczytko and Stewart 1979) or region (e.g., Nimmo 1971, Baumann *et al.* 1977, Ward and Kondratieff 1992) and are generally available as journal publications or guide books. Detailed, species-specific identification may require consultation with specialists and occasionally rearing of aquatic insects to the adult stage.

III. SPECIFIC METHODS

A. Basic Method 1: Distributions and Habitat Relationships

If you carefully examine a reach of stream, you will discover that many populations of lotic macroinvertebrates are not distributed uniformly throughout the reach. Rather, species tend to be found in particular hydrogeomorphic habitats (see Chapters 4 and 7). Habitat-specific distributions may be found at fairly large scales (e.g., riffles, pools, runs, woody snags, backwaters; see Chapter 2) or at very small scales of resolution (e.g., bottoms compared to tops of stones, along points of laminar flow, see Chapter 4). This exercise is designed to introduce the concepts of abundance and diversity within the stream macroinvertebrate community and how these features may differ among habitats within a

single stream reach. You will be using the methods described previously in *Field Sampling* to obtain quantitative samples from a variety of readily identifiable stream habitats.

Laboratory Preparation

1. One set of field collecting gear (listed below) should be available for every two field researchers working together as a team.
2. Select an appropriate stream segment from the study stream. This may be based on knowledge that you have acquired from other fieldwork or specifically from work associated with earlier chapters in this book.

Field Collection

1. At streamside, identify several different habitat types along a stream length approximately equal to 10 times the width of the stream. Within this segment, you should be able to discriminate among several different habitats.
2. Sketch a simple diagram of the stream reach that you are going to sample (see Chapter 4 for an example of method and detail; also see Chapter 7).
3. If this site has been used for other exercises, refer to your notes regarding patterns of current velocity, substratum size, channel cross-section, spatially explicit habitats and large woody debris.
4. Delineate and note the range of microhabitats present.
5. Enter the stream and carefully look for macroinvertebrates on cobble, rock out-crops, large wood debris, or other hard surfaces. Make notes concerning your observations.
6. *Special Note*: Some species of macroinvertebrates have very narrow microhabitat requirements and/or may achieve very high densities when environmental conditions are favorable. For example, look closely for black fly larvae (Diptera: Simuliidae). Black fly larvae generally have well developed "fans" on the head used for straining food particles from the stream current (Parkes *et al.* 2004). Because they have narrow flow requirements, black flies often occur in very high abundance in very specialized microhabitats that have a stable substratum and smooth-laminar flows (Malmqvist *et al.* 2001). Black fly larvae may exclude other larvae from areas around them by nipping and biting; this often results in uniform spatial distribution patterns. Look for these and other macroinvertebrates that may occur in easily observable areas and note similarities and dissimilarities of microhabitat.
7. Take a sample from each of the different habitat types that you identified. Use a Surber, Hess, Stanford-Hauer kicknet, or D-frame sampler, depending on the type(s) of habitats that are present.[3]
8. Empty the contents of the sampler into a 20-L plastic bucket. Examine each sample for the presence of macroinvertebrates while the sample is in the bucket. At this juncture you must decide whether samples will be returned to the laboratory for detailed examination or sorted in the field. If the samples are to be preserved immediately and returned to the laboratory for further analysis, go to step 9. If samples are to be processed in the field, go to the next section on field sorting.

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[3] Collection, sorting and identification of organisms may take several hours per sample; thus, if this exercise is being used within a class setting, we recommend a careful examination of time allocation.

9. Pour the sample contents from the 20-L bucket into a sieve and let the water drain from the sample. Transfer the sample into an appropriate sized container (e.g., 500-mL jar or 1-qt Ziplock bag), place a label containing date, site, and sample number into the container (use a small piece of paper and pencil), and preserve with 95% ethanol to cover the sample completely and to reach a final concentration of 70% ethanol.[4]

Field Sorting and Identification

1. If samples are to be sorted and identified at streamside, pour the contents of the sample from the bucket into an appropriate meshed sieve (e.g., 125 μm, 250 μm, or 500 μm). Refloat the sample by immersing the sieve in water (filling the enamel pan used in the next step with stream water, being cautious not to let extraneous organisms into the sample, is a convenient way to do this), being careful not to allow the water to breach the upper lip of the sieve and thereby lose sample contents. Distribute the sample evenly on the sieve-screen and remove the sieve from the water.

2. Using a spoon or butter knife, divide the sample into approximately four equal sections on the surface of the sieve-screen. You have now divided your sample into 1/4 subsamples. Remove one of the 1/4 subsamples, place it into a white enamel pan, add stream water, and distribute the sample around the pan. You should be able to observe many macroinvertebrates crawling or swimming about the pan.

3. It is important to have a sufficient sample size (i.e., one consisting of several hundred individuals). If there are tens of individuals in the pan, then add additional 1/4 subsamples from the sieve, as needed. If there are thousands of individuals, then you will need to further subsample by taking the 3/4 sample remaining on the sieve, returning it to the 20-L bucket and placing the current contents of the 1/4 subsample in the pan back into the sieve. Now go back to step 1 and reconduct the subsampling procedure. Remember that now the subsamples are 1/16 of the original sample.

4. While invertebrates are still in the enamel pan, examine the body shape of the benthic animals you see from the different microhabitats.

5. Using forceps, remove all macroinvertebrates from the sample (or subsample) and sort them into easily recognized groups. At a minimum, you should be able to identify taxa to the phylum- and order-level using the general key provided in Appendix 20.1.

6. Place each taxon in a different container.[5] Count and record the number of individuals within each taxon.

7. For detailed identification and enumeration, the sorted sample must be returned to the laboratory and examined using a binocular dissecting microscope. Place each sorted taxon into a separate container (e.g., scintillation or other glass vial), record the date, site, and sample number on a label for each container using a piece of paper and pencil (do not use a pen; most inks will fade to illegible in ethanol), and preserve with 70% ethanol.

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[4] Add a small amount of rose bengal to the sample to stain invertebrates and aid in the separation of organisms from debris.
[5] Wells of a muffin tin, plastic ice-cube tray, or styrofoam egg carton work well for this purpose.

Laboratory Sorting, Identification, and Enumeration

1. If samples were not field-sorted, use the laboratory sink and empty the contents of a sample into an appropriate meshed sieve (e.g., 250 μm or 500 μm). Rinse the sample thoroughly with tap water being careful not to lose any material. Go through the subsample and sorting procedures described in steps 1–5 above in "Field Sorting and Identification." Be sure to dilute the used ethanol by at least 10× as you dispose of it in the sink.
2. Use the key provided (Appendix 20.1) to identify the most commonly occurring taxa to the family level for insects and class level for non-insects. If the organism you are identifying does not "key out" or you desire greater resolution in identifications use McCafferty (1981), Merritt and Cummins (1996), Pennak (1989), and Thorp and Covich (2001) to separate the various taxa. A binocular dissecting microscope will be needed to view the morphological structures that are used to identify the organisms.
3. *Special Note*: It will not be possible for the beginning student to identify in a single laboratory exercise all the various organisms that are typically found in an unpolluted stream. It generally takes months of work to develop the skills to identify organisms to the generic level and a lifetime of work to the species level.
4. Observe the diversity of species within these taxonomic groups.
5. Select one or two taxonomic groups to examine in detail. For example, you may select to look at the net-spinning caddisflies (superfamily Hydropsychoidea, especially the family Hydropsychidae) or the predaceous stoneflies in the family Perlidae.
6. Carefully sort and identify the individuals from the taxa that you have chosen to study.
7. List the genera/species collected from each of the different habitats. If identification beyond the taxonomic level presented in Appendix 20.1 is beyond your current expertise, then genera/species found within your chosen taxon may be further separated as species A, B, C, and so on.

Data Analyses

1. Enumerate the selected taxa from each sample collected.
2. Calculate mean density and standard deviation for each selected taxon by habitat.
3. These data for the macroinvertebrate assemblages (e.g., abundance, taxa richness) can be used to calculate various population descriptors. Calculate two common diversity indices that are relative measures of species richness and equitability: the Shannon-Wiener index and Simpson's index. The Shannon-Wiener information theory index (H′) is calculated as:

$$H' = -\sum p_i \log p_i \qquad (20.1)$$

where p_i = proportion of the total number of individuals in the ith species.

Simpson's index (λ) is the probability that any two individuals picked at random will be of the same species and is calculated as:

$$\lambda = \sum p_i^2 \qquad\qquad (20.2)$$

were p_i is as above.

Simpson's index is a measure of the extent that individuals in a sample are concentrated into a few species. See Chapter 31 for biotic indices used with stream macroinvertebrates.

Special Note: Sorting, identification, and enumeration of macroinvertebrate samples can be very time consuming for even the most accomplished aquatic entomologist or benthic ecologist. Merritt *et al.* (1996) provide an excellent flow diagram summarizing the general procedures for analyzing benthic samples. Depending on taxonomic complexity, abundance, and extent of analyses (e.g., enumeration, wet weights, ash-free dry mass) a single sample may take 8–10 hours (or more) spread over several days to completely sort and identify.

B. Basic Method 2: Watershed Scale Distribution

Stream ecologists have noted that particular macroinvertebrate species often occur only within very restricted stream reaches. In some cases, this is because the habitats that particular species require only occur within certain well-defined reaches. Although various habitat types occur along the entire length of the stream (e.g., riffles), one will find many species that are reach-specific along the river continuum. This exercise is designed to illustrate the macroinvertebrate species-replacement that can occur along the downstream gradient of a river network. In this exercise, we will collect macroinvertebrates from riffles, runs, backwaters, and other habitats that can be differentiated following the protocols outlined in Chapters 2, 4, and 7. Depending on your specific research question, you may examine the macroinvertebrate assemblages from each stream reach looking for changes in species composition with an emphasis on closely related species, or you may decide to distribute your effort across the entire macroinvertebrate assemblage. In the event that this is an exercise to be accomplished by a class or special research project, we suggest that you focus your attention on the net-spinning caddisflies (Trichoptera: Hydropsychoidea) for this exercise, because they occur in almost all unpolluted running water systems, especially in riffles of gravel-bed streams or on stable substrata (e.g., woody snags) in sandy bottom streams or rivers. Also, the net-spinning caddisflies are easily recognized and larval taxonomy is fairly well known for many areas of North America and Europe.

Laboratory Preparation

1. Select a forth- to fifth-order stream network using a detailed watershed map(s) (e.g., USGS quadrangle map, scale 1:24,000).
2. Select a series of sampling sites along the stream-river longitudinal corridor, with consideration for ease of access and diversity of habitats among and within sites.

A site should be chosen to represent each order of stream. The researcher should be well versed in advance of going to the field, carefully considering the number of replicate samples to be taken within each of the habitat types identified following a habitat mapping exercise at each site (see Chapter 4).

3. Consider the various physical and biological variables that may affect macroinvertebrate distribution and abundance. Determine which of these factors are to be investigated along with the collection of the benthic samples. Following the protocols for mapping (Chapter 4), collecting velocity data (Chapters 3 and 7), temperature (Chapter 5), groundwater/surface water interactions (Chapter 6), or other physical and biological variables, lay out a strategic plan for gathering and recording the data.

Field Collection

1. Obtain quantitative samples from each habitat using the technique described above in *Field Sampling* of Basic Method 1 (e.g., a Stanford-Hauer kicknet, Surber, or Hess sampler) for riffle habitats and dip-net samplers for backwaters areas where the current is not sufficient to transport dislodged macroinvertebrates into the net.
2. If this is rapid examination exercise, examine each sample for the presence of the macroinvertebrate guild of interest (e.g., hydropsychid caddisflies, perlid stoneflies, or ephemerellid mayflies). Place contents of the sample into a white, enamel pan and use forceps to remove the specific taxa of interest from the sample material. Place larvae into a suitably sized plastic jar with 70% ethanol, using a single jar for each sample. The remainder of the live sample may be preserved for additional examination or returned to the stream.
3. If this is a comprehensive study, collect all organisms, place them into a sieve or small net to remove excess water, and place the contents of the sample into a plastic jar with 70% ethanol, again using a single jar for each sample. Be certain to label the sample jars by placing a label affixed to the outside of the jar and on a piece of paper inside the jar.
4. Collect all relevant biological and physical data for each sample collected (e.g., riparian vegetation, substratum characteristics, current velocity, depth, etc.).

Laboratory Analysis

1. After bringing samples back to the laboratory, identify target taxa to the genus- or species-level. Use a binocular dissecting scope to examine the organisms and their key morphological structures. Merritt and Cummins (1996) provide generic-level keys; however, various regional keys for identification of species are available. (For illustrative purposes, you can use "morpho-species"—e.g., species A, B, C, etc.).
2. Observe the morphological diversity and taxa richness of species within the taxonomic and functional group(s) with which you are working.
3. Carefully sort, identify, and enumerate the individuals of each taxon from each sample.
4. Calculate the abundance for each species by sample and determine means, standard deviations, and Shannon-Wiener and Simpson's indices by site and by habitat.

C. Advanced Method 1: Population Dynamics and Movement

Populations change in size over time, increasing from new births and the immigration of individuals from other areas, and decreasing from death and emigration. In this exercise, we will mark members of populations of aquatic insects to observe their movements over time, as well as losses (emigration, death) or gains (immigration, births) to the population (see also Chapter 21 for a more detailed discussion of marking stream invertebrates).

Water striders (Family Gerridae, and the species you probably have is *Aquarius* (=*Gerris*) *remigis*) occurs on several continents; *A. remigis* likely is the most widely distributed species of aquatic insect worldwide. Individuals commonly occur in the slow-flowing margins and pools of streams [see Spense and Anderson (1994) for a detailed review of the biology of water striders]. Using a handnet, catch one of these surface-dwelling creatures. Note that its "back" (i.e., the dorsum of the thorax) is where we will apply our marking tag: typewriter correction fluid, which comes in a variety of colors, and by using up to three marks on an insect and different colors, scores of individuals can be marked and followed.

Behavioral Observations

1. Working in pairs, we will describe the spatial distribution of *A. remigis* (or some other insect such as a cased limnephilid caddisfly such as *Dicosmoecus*; see below).
2. Map a segment of stream (∼50-m reach) following the example of Chapter 2 or 4 (or if you are working in the same location, use the maps created in an earlier exercise).
3. Collect and sex (in the case of water striders) each individual (seeing the two genders side by side makes this pretty clear); then mark them by using different colors and release them where you caught them.
4. Observe the behavior of individual water striders with respect to their location in the stream, and their resting, mating, searching, fighting, and feeding behaviors. Watch each individual for 10–15 minutes, and be sure to compare individuals of different sex and maturity.
5. Make detailed notes on each of these behaviors and the time they spent doing each activity.

Mark and Recapture

1. A final exercise is a mark-recapture study (see Chapter 22 for detailed rationale and assumptions underlying mark-recapture studies).
2. This method involves sampling on two days, about one week apart.
3. Record the number of individuals originally marked on day 1, the number collected on day 2 that were marked and unmarked, and then calculate population size (N) as follows:

$$N = \frac{M \times C}{R} \tag{20.3}$$

where M = number originally marked, C = total catch on day 2, and R = the number of day 2 recaptures (i.e., those originally marked on day 1).

4. *Special Note*: The larger cased caddisflies in the family Limnephilidae (e.g., *Dicosmoecus* spp.) are also appropriate for this type of study (see Appendix 20A). To mark each individual, remove the larva and case from the water, pat the case dry, add the mark (use colored, permanent-marker pens), and then return the caddisfly to the stream at the place collected. Another interesting exercise is to compare upstream-downstream movements of marked larvae that have all been released at a single point.

D. Advanced Method 2: Laboratory Artificial Stream Experiments

Many experiments that can be conducted in laboratory streams with aquatic macroinvertebrates (see Hauer 1993). Lamberti and Steinman (1993) provide many designs and applications of laboratory streams. In this exercise, we will construct several small air-lift chambers that provide the microhabitat-flow requirements needed by black flies (Simuliidae) and determine larval growth rates under different environmental conditions. See Hauer and Benke (1987) for detailed methods and the construction and operation of these small artificial stream tanks.

Setup and Experimentation

1. Construct at least four artificial stream tanks.
2. Using tropical fish aquarium supplies (listed below) arrange the artificial stream tanks to provide an "airlift" current when the air pump is on (see Hauer and Benke 1987).
3. Water level in the artificial stream tanks should be maintained near full, but not so full that water spills over the top.
4. Obtain black fly larvae from a nearby stream (typically found in shallow, high-velocity habitats) and return live specimens to the laboratory in a large bucket. Collect at least 200–250 individuals in midsize classes (3–4 mm).
5. Remove a random sample of 15–20 animals of the population and preserve in 70% ethanol.
6. Distribute the remaining animals randomly among the four artificial stream tanks. Maintain tanks and permit larvae to feed and grow over a one- to two-week period. You will need to add replacement stream water to the tanks twice daily throughout the experiments to maintain water levels and natural food levels. This is best done by bringing unfiltered stream water to the laboratory in 20-L carboy containers. Keep track of the amount of water added over the duration of the experiments.
7. Experimental conditions may be varied among the artificial stream tanks. For example, some tanks may be kept at cool temperatures in a refrigerator or environmental chamber while other tanks are maintained at room temperature. Likewise, some tanks may be given a supplemental food source of either cultured algae, natural seston collected from a stream, or small quantities of granular baker's yeast.

Analysis of Growth Experiments

1. Terminate the growth experiments after 10–14 days or as soon as the first individuals begin to pupate, whichever comes first.
2. Keeping larvae from each tank separate, collect and preserve animals in 70% ethanol.

3. Using a dissecting microscope fitted with an ocular micrometer, measure the total length of all larvae from each experimental stream tank and the larvae that were preserved at the start of the experiments.
4. Dry mass (*DM*) of each larva may be predicted from the regression (Hauer and Benke 1987)

$$DM = 0.0031 \times BL^{2.64} \tag{20.4}$$

where BL = body length in mm.
5. Calculate daily instantaneous growth rates (*g*) for larvae as:

$$g = \frac{\ln(DM_f/DM_i)}{t} \tag{20.5}$$

where DM_f = mean dry mass (in mg) of larvae at the end of the growth experiment, DM_i = mean dry mass of the larvae at the start of the experiment, and t = number of days for the particular trial.

IV. QUESTIONS

1. Did you observe specific macrohabitat preferences (e.g., riffle, pool, backwater)? Did you observe specific microhabitat preferences (e.g., top, side, or bottom of rock within a riffle)? How do these relate patterns to the morphological and behavioral adaptations described in the Introduction?
2. Were you able to see morphological differences among the species that you collected from different habitats? How did the morphology of species collected from riffles differ from species collected from pools, debris dams, or leaf litter?
3. Consider the breadth of different habitats that you have observed in stream ecosystems. Imagine that you are standing next to a stream whose bottom and sides are concrete. In your mind or on paper consider how you would remake this concrete channel into a living stream. What structural components would you add to increase microhabitat complexity (and hence abundance of organisms)? Consider the stream bed. How would you integrate the hyporheic zone into your imagined stream? Where do factors such as riparian vegetation and nutrient sources come into play? You've now begun to think about stream restoration.
4. Did you observe distinctly different species within the larger taxonomic group that you identified? Even though you may not have been able to identify your specimens to the species level, how many different putative taxa were you able to distinguish?
5. Did you observe a pattern of different species among sample sites along the longitudinal gradient of the stream within the taxonomic group that you studied in detail? What general patterns of species distributions or replacements did you observe?
6. How do water striders respond to differences in flow? On your original map, record areas of fast, medium, and slow flow (see Chapter 4) and compare to strider

distribution. Did sex, age, or other factors within the water strider experiments appear to influence distribution? Can food resources you provide be used to alter microhabitat selection? What are some assumptions that we make about the effect of the mark on the animal when we conduct such experiments?

7. If you conducted growth experiments, what was the growth rate of black fly larvae from each of the experimental stream tanks? Did different temperatures or different levels of food resources affect growth rate?

8. Consider instantaneous growth (g). What relationship does g have to secondary production? (see Chapter 29).

V. MATERIALS AND SUPPLIES

Field Materials

> 20-L plastic bucket
> <125-μm sieve or small bag-net
> 95% ethanol
> Current velocity meter
> D-frame net or Stanford-Hauer kicknet
> Meter sticks
> Permanent marker pens (variety of colors)
> Stop watch
> Typewriter correction fluid (various colors; for Advanced Method 1)

Laboratory Equipment and Materials

> 70% ethanol
> Appendix 20.1 and reference books mentioned in text
> Artificial stream tank, air pump, tubing (for Advanced Method 2)
> Binocular dissecting microscope
> Forceps
> Scintillation vials or patent-lip vials with neoprene stoppers
> White enamel pans for sorting

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Appendix 20.1

A simplified key for the rapid identification of the most common stream macroinvertebrates. Noninsect taxa are described to the phylum or order level. Insect taxa are described to the family level. Many more stream macroinvertebrates occur than are presented here; this key is intended to only serve as a starting point for their identification. (Some illustrations taken from Betten 1934, McCafferty 1981, and Thorp and Covich 2001, with permission.)

**General Key to Common
Stream Macroinvertebrates**

KEY 1

Soft-bodied with a hard
calcareous shell
(Mollusca)

Body not enclosed in a
hard calcareous shell

Body enclosed in two
hinged shells (Bivalvia)
clams and mussels

Adults and most larvae
with segmented legs.
Larvae without legs have
prolegs and/or mandibles
(Arthropoda)

Body enclosed in
a single shell

Shell in a whorl
(Gastropoda)
snails

Legs absent in
all life stages

Go to KEY 2

Shell not in
obvious whorl
(Gastropoda)
limpets

Unsegmented
flat worms
(Turbellaria)

Shell whorl planar
(Planorbidae)

Shell whorl
spiraled

Segmented worms

With spire pointing up,
aperture on the right
(Lymnaeidae)

With spire pointing up,
aperture on the left
(Physidae)

Segmented worms
without suckers
(Oligochaeta)

Segmented worms with
anterior and posterior
suckers (Hirudinea)
leaches

**POSTERIOR
SUCKER**

KEY 2

Arthropoda
(from KEY 1)

Two pair of antennae, variable number but more than three pairs of legs (Crustacea)

First three to five pairs of thoracic legs modified as maxillipeds; may include large chelae (Decapoda) crayfish and shrimp

No legs modified as maxillipeds

One pair of antennae, generally with three pairs of legs, but may be absent. Adults generally winged (Insecta)

Seven pairs of legs, body flattened dorso-ventrally (Isopoda) sow bugs

Five pairs of legs, body laterally flattened (Amphipoda) scuds

Thorax with three pairs of segmented legs Go to KEY 3

Thorax without segmented legs. Prolegs or pseudopods often present (Diptera) true flies

Larvae with what appear to be 7 segments, ventral surface has 6 prominent sucker-discs (Blephariceridae) net-winged midges

Larvae with more than 7 segments, and without sucker-discs

Larvae with obvious sclerotized head capsule

Larvae without obvious sclerotized head capsule or head is retracted into thorax

Body shaped like a bowling pin, head with fanlike feeding structures (Simuliidae) black flies

Body long and cylindrical, without fan like feeding structures (Chironomidae) midges

Head capsule retracted into thorax, body without ventral prolegs (Tipulidae) crane flies

Some external head structure visible, body with ventral prolegs (Empididae) dance flies

**Insecta
(from KEY 2)**

KEY 3

**Insects with large
functional wings**

**Wingless or with
developing wings (wing
pads) on thorax**

**Front pair of wings
hard and shell-like
(Coleoptera)
adult beetles**

**Front pair of wings
"leathery" and
hard at the basal
half (Hemiptera)
adult "bugs"**

**Generally without
developing wingpads
Go to KEY 6**

**Generally with
developing
wingpads**

**Posterior end of abdomen with 2 or
3 many-segmented cerci (tails),
without hinged mouthparts**

**Posterior end of abdomen
without many-segmented
cerci, with hinged mouthparts
(Odonata)**

**Abdomen with leaf-like or
feather-like gills, legs with a
single tarsal claw, generally
but not always with three
cerci (Ephemeroptera)
mayfly nymphs
Go to KEY 4**

**Abdomen without gills. If
gills are present they appear
as filaments along thorax or
at end of abdomen, legs with
two tarsal claws, never more
than two cerci (Plecoptera)
stonefly nymphs
Go to KEY 5**

**Nymph with 3 leaf-like
gills at the end of the
abdomen (Zygoptera)
damselflies**

**Nymph without 3 leaf-
like gills at the end of
the abdomen
(Anisoptera) dragonflies**

**Hinged
Mouthpart**

**Hinged
Mouthpart**

KEY 4

Ephemeroptera mayflies (from KEY 3)

Enlarged thoracic notum forming shield covering gills (Baetiscidae)

No thoracic shield, abdominal gills exposed

Head without anteriorly projecting tusks

Head with anteriorly projecting large tusks visible from above (Ephemeridae)

Gills on abdominal segment 2 large and operculate

Gills on abdominal segment 2 absent, or if present not operculate

Operculate gills on abdominal segment 2 not separated

Nymphs generally robust, gills absent from abdominal segment 2 (Ephemerellidae)

Operculate gills on abdominal segment 2 well separated (Leptohyphidae)

Operculate gills on abdominal segment 2 fused along midline (Neoephemeridae)

Operculate gills on abdominal segment 2 overlapping (Caenidae)

Nymphs generally delicate, gills present on abdominal segments 1–7 or 2–7

Head and body fusiform, eyes distinctly along outside edge of head when viewed from above

Head and body distinctly flattened, on dorsal surface of head (Heptageniidae)

Gills forked or ending in filaments (Leptophlebiidae)

Gills as single or double lamellae

Middle cerci (tail) reduced or absent, if present then antennae 2–3 times head width (Baetidae)

Three well developed cerci (tails), antennae shorter than 2 times head width (Siphlonuridae)

**Plecoptera
stoneflies
(from KEY 3)**

KEY 5

**Nymph "roach-like";
thoracic sterna appear
as large plates
(Peltoperlidae)**

**Nymph not "roach-like";
thorax not as large plates**

**Branching gills at the
base of the legs**

**No branching gills at
the base of the legs**

**Branching gills only at the
base of the legs, never on
abdominal segments 1 or 2;
gills may be present at
posterior of abdomen
(Perlidae)**

**Branching gills at the base
of the legs, and abdominal
segments 1 and 2
(Pteronarcyidae)**

**Mouthparts with 3
distinct notches formed
by the glossae and
paraglossae being of
equal length**

**Mouth-parts with a
single wide notch formed
by the paraglossae
positioned well forward
of the glossae**

Paraglossae

Glossae

**Thorax robust, with
divergent wing pads**

**Thorax slender, with
parallel wingpads**

**Wingpads parallel
forming a distinct U;
cerci (tails) shorter
than abdomen
(Chloroperlidae)**

**Lateral fold along
abdomen only
extending about half
way and no farther
than segment 7
(Leuctridae)**

**Wingpads divergent
forming a distinct V;
cerci (tails) as long or
longer than abdomen
(Perlodidae)**

**Tarsal segments 1 and 2
about the same length
(Taeniopterygidae)**

**Entire length of
abdomen divided
by a lateral fold
(Capniidae)**

**Tarsal segment 2 much
shorter than segment 1
(Nemouridae)**

(from KEY 3)

KEY 6

Abdomen with well developed lateral filaments

Abdomen with lateral filaments or may have dorsal filaments also, never with anal prolegs, caudal appendage paired or absent but never single (Coleoptera) beetle larvae

Abdomen without lateral filaments, but may have lateral gills

Abdomen with lateral filaments and 4 stout hooks at end (Gyrinidae) whirligig beetles

Abdomen with long dorsal filaments (Haliplidae) crawling water beetles

Abdomen always ends in a pair of prolegs, each with a single hook, antennae 1-segmented and inconspicuous; no abdominal filaments as shown but may appear as single or tufts of gills (Trichoptera) Go to KEY 7

Abdomen with 7 or 8 pairs of single lateral filaments, segment 9 with either anal prolegs each with 2 hooks, or a single medial anal filament (Megaloptera)

Abdomen ends variously, but never in a pair of prolegs (Coleoptera) beetle larvae

Body very broad, oval, and flattened, head and legs hidden when viewed from above (Psephenidae) water pennies

Abdomen terminating in a single long filament (Sialidae) alderflies

Abdomen terminating in 2 prolegs with 2 hooks each (Corydalidae) dobsonflies

Body elongate, head visible from above

Mandibles elongate and pointed for grasping and piercing prey (Dytiscidae) predaceous water beetles

Mandibles smaller, underside of first 6 segments with a shallow groove on each side (Elmidae) riffle beetles

**Trichoptera
caddisflies
(from KEY 6)**

KEY 7

**Larval body strongly
curved, case of small
grains in spiral shape
resembling a snail case
(Helicopsyche)**

**Each of 3 thoracic segments
covered by a single large
sclerotized dorsal plate**

**Larval body not strongly
curved, case if present
not snail shaped**

**Third thoracic segment
(metanotum) mostly membranous,
may have small sclerotized plates
and /or setal hairs**

**Abdomen with rows of
branched gills, larvae
construct a retreat with
a silk capture net
(Hydropsychidae)**

**Abdomen without branched gills,
larvae construct a case of various
shapes, final larvae size very small
(Hydroptilidae)**

**Antennae long, at least
6 times as long as wide,
OR pair of narrow curved
sclerites on middle thoracic
segment, case is variable
(Leptoceridae)**

**Antennae short, not more than
3 times as long as wide, mesonotal
sclerites never curved**

**Second thoracic segment
(mesonotum) with single or
connected sclerotized plates
Go to KEY 8**

**Second and third thoracic
segments (meso- and
metanotum) entirely
membranous**

**Anal claws very small and oriented
at right angle to the length of
abdomen, case turtle-shaped
(Glossosoma)**

**Most of anal proleg long,
free and well-developed**

**Abdominal segment 9
entirely membranous**

**Labrum scerotized,
not T-shaped**

**Abdominal segment 9
with well-developed
sclerotized plate
(Rhyacophilidae)**

**Trochantin of first
thoracic leg broad and
hatchet-shaped,
larvae construct
tubular-shaped nets
(Psychomyiidae)**

**Trochantin of first
thoracic leg pointed at
apex, larvae construct
funnel-shaped nets
(Polycentropodidae)**

**Labrum membranous and
T-shaped, larvae construct
sack-shaped filter nets
(Philopotamidae)**

KEY 8

**Trichoptera
caddisflies (cont.)
(from KEY 7)**

**Pronotum divided by a deep furrow, no
dorsal or lateral humps on abdominal
segment 1; case often square in
x-section but may be round
(Brachycentridae)**

**Pronotum not divided by furrow, first
abdominal segment with lateral hump
and usually also dorsal hump**

**Antennae located about midway between
eye and mandibles; median dorsal hump
present on abdominal segment 1**

**Antennae located extremely
close to the eye; median
dorsal hump absent; case
variable of wood or stones
(Lepidostomatidae)**
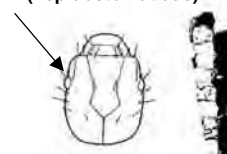
**Mesepisternum formed
anteriorly into a sharp
elongate process
(Goeridae)**
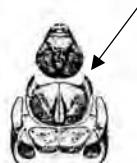
**Mesepisternum not formed
into a sharp elongate process**

**Notch along the mesal suture
(midpoint along anterior
edge) of the mesonotum
(Uenionidae)**
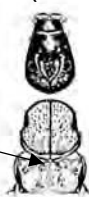
**No notch along the mesal
suture of the mesonotum**

**Mandibles not modified into scraping
blades; cases are highly variable of plant
and mineral materials and in many
shapes; many of the larvae in final instar
prior to pupation are large (>12 mm)
(Limnephilidae)**
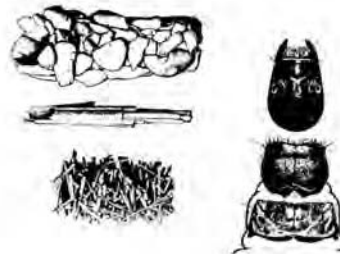
**Mandibles with uniform
scraping blades; case usually
cornucopia shaped
(Apataniidae)**
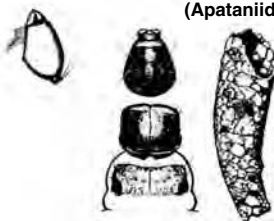
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Macroinvertebrate Dispersal

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I. INTRODUCTION

Dispersal, or the movement of individuals from one area or habitat patch to another, is an activity exhibited by most aquatic invertebrates (Bilton *et al.* 2001). Dispersal occurs at rates that are species-specific, is an integral aspect of the population dynamics of species, and provides gene flow that affects the genetic structure of populations and helps maintain genetic diversity within and among populations. Dispersal also is a key process in the recolonization of disturbed areas of streams, such as in sediments scoured and denuded by spates. Both active and passive dispersal movements are common among stream-dwelling benthic invertebrates in response to a number of factors. The continuous flow of water in lotic environments provides a convenient and energetically efficient mechanism for downstream dispersal, but it can cause unwanted displacement of individuals to downstream areas and can make upstream dispersal difficult. Overland dispersal also occurs, primarily through the flight of adult insects that emerge from streams.

This chapter introduces key concepts and sampling methods concerning the dispersal of stream-dwelling, benthic invertebrates. Methods are provided to quantify downstream drift, the movements of individual organisms, and the emergence and flight of adult insects. Methods also are provided to examine aspects of the movements of benthic invertebrates associated with their colonization of substrata in streams. Finally, procedures are discussed concerning the use of population genetic approaches to the study of the dispersal of benthic invertebrates.

A. Drift

Although most invertebrates that occur in streams and rivers are benthic, a net placed in the water column often will collect many individuals. These organisms are drifting, an activity whereby they enter the water column and are transported downstream by the current. *Drift* is one of the most important mechanisms for the dispersal to and colonization of downstream habitats by a wide variety of stream invertebrates. Drift also is one of the most studied activities of benthic fauna and has been the subject of a number of reviews (Waters 1972, Müller 1974, Wiley and Kohler 1984, Brittain and Eikeland 1988, Allan 1995).

An interesting aspect of drift, and one that has intrigued stream ecologists for decades, is that drift usually exhibits a distinct diel periodicity whereby the number of individuals drifting changes over a 24-hour period. The majority of species drift in maximum numbers sometime during the night. The most common drift pattern is that of highest numbers drifting shortly after sunset (Figure 21.1), although peaks in the middle of the night and just prior to sunrise also occur.

Whereas some individuals may passively enter the drift, for example by accidentally being swept away by the current (Kovalek 1979, Wilzbach *et al.* 1988), others exhibit what is known as drift behavior, or active entry into the water column (Wiley and Kohler 1984). Changes in ambient light intensity, although not the ultimate reason for drift behavior, serve as the trigger or phase-setting agent for drift. Most species have a threshold light intensity below which active drift may be initiated, as occurs at sunset (Allan 1995). The distance drifted by individuals varies depending on species-specific behavior and stream hydrology and structural habitat features (Lancaster *et al.* 1996, Holomuzki and Van Loan 2002) but can range from a few cm to 10's of meters.

A variety of reasons for invertebrates to actively drift have been suggested. These include dispersal in search of suitable resources such as food and substratum (Walton *et al.* 1977, Hershey *et al.* 1993, Koetsier *et al.* 1996, Siler *et al.* 2001), escape from predators and competitive interactions (Flecker 1992, McIntosh *et al.* 2002), avoidance of unfavorable environmental conditions including various forms of pollution (Wallace *et al.* 1989, Schulz and Liess 1999), and movements associated with life history events
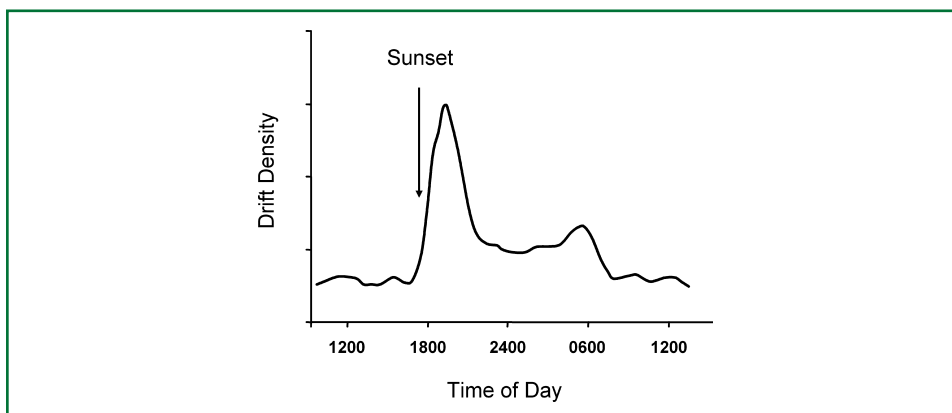
FIGURE 21.1 Typical macroinvertebrate diel drift pattern.

such as egg hatching, pupation, and emergence (Otto 1976, Krueger and Cook 1981, Ernst and Stewart 1985).

While there are many potential benefits of drifting, there are also potential costs. One of those is the increased risk of capture by fish, as invertebrates are very vulnerable to fish predation while drifting. Many stream-dwelling fish, however, are size-selective visual feeders, needing to see their prey to capture them and preferentially preying on larger individuals. The propensity of invertebrates to exhibit nocturnal drift thus increases as the risk of predation by visual-feeding fish increases (Flecker 1992, Forrester 1994, March *et al.* 1998). Risk of predation also can affect the size distribution of drifting invertebrates, with larger individuals being more prone to drift at night (Allan 1978, 1984).

Methods are provided in this chapter to (1) demonstrate the general sampling techniques used to quantify drift and (2) illustrate differences in the numbers and size composition of invertebrates drifting between day and night.

B. Colonization and Movement

Colonization is a process whereby organisms disperse to and become established in new areas or habitats, or in disturbed habitats in which they previously were present (more accurately called *recolonization*). It occurs over broad and variable spatial and time scales (Sheldon 1984, Mackay 1992) and includes sediment surfaces as well as other substrata such as woody debris (Thorp *et al.* 1985). Colonization of new or denuded substratum is a common phenomenon in streams, occurring primarily as a response to sediment-scouring storms, the input of new woody debris, and disturbances such as toxic pollutants and drying of the streambed during periods of drought.

Numerous studies have shown that invertebrates can quickly colonize new or disturbed substrata, although the rate of colonization differs among species and with distance from colonizing sources, time of the year, and the physical characteristics of the substratum (Williams 1980, Gore 1982, Lake and Doeg 1985, Peckarsky 1986). Particle size is an important factor in determining community structure in streams (Minshall 1984) and many species have morphological or physiological adaptations suited to their preferred substratum. These preferences help determine the likelihood of an individual dispersing to, colonizing, and remaining on specific substrata.

Colonization of substrata requires dispersal of organisms from source areas to the new or disturbed substratum. Williams and Hynes (1976) noted four routes of colonization for invertebrates. First, downstream movement of organisms, primarily by drift, is typically considered as the most important in-stream method of movement and hence colonization. Second, upstream movements along the sediment, possibly the result of a positive rheotaxis by invertebrates, also occur (Söderström 1987, Delucchi 1989, Mackay 1992). Although often considered of minor importance to the colonization process, crawling and swimming of organisms may be an underappreciated but important mode of movement for aquatic invertebrates both within the channel (Doeg *et al.* 1989, Giller and Cambell 1989, Humphries 2002) and to surrounding floodplains (Smock 1994, Huryn and Gibbs 1999).

Third, movements to and from the subsurface, or hyporheic zone, have also been documented in streams (Benzie 1984, Delucchi 1989, Boulton *et al.* 1991; see also Chapters 6 and 33). The hyporheic area of streams can have high densities of a wide variety of invertebrate species and may serve as a refuge for organisms during unfavorable conditions on the surface (Boulton 1988, Sedell *et al.* 1990, Clinton *et al.* 1996).

It thus may be an important source of animals for colonizing surface substrata following disturbances.

Finally, colonization by aerial sources is a potentially important mechanism of colonization in all streams (Gray and Fisher 1981, Benzie 1984, Cushing and Gaines 1989, Mackay 1992). Ovipositing (i.e., egg-laying) by aerial adults is the primary mechanism of aerial colonization, though colonization by flying adults of coleopterans and hemipterans also occur. The magnitude and rate of aerial colonization at a given location is greatly affected by the time of year and the distance from source areas.

Methods are provided in this chapter to (1) quantify invertebrate movements and the process of colonization over time; (2) determine the effects of sediment particle size preferences on colonization by invertebrates; (3) demonstrate the different routes of movement and colonization in streams; and (4) follow the movements of individual invertebrates over time.

C. Adult Emergence and Dispersal

The sampling of aquatic insects has historically focused on their immature stages. Most species of aquatic insects, however, metamorphose into adults that emerge from the water and are then active in the terrestrial environment. While a primary activity of adults is mating and ovipositing, most individuals are capable of dispersal flights that can result in the laying of eggs far from the site of emergence. Besides facilitating colonization of new areas and habitats, these flights also affect genetic diversity by providing gene flow across drainages and among subpopulations of species. The majority of studies on the genetic structure of aquatic insect populations, however, have shown considerable genetic heterogeneity at various spatial scales, suggesting somewhat limited dispersal and hence gene flow (Schmidt *et al.* 1995, Bunn and Hughes 1997, Kelly *et al.* 2002, Miller *et al.* 2002).

Studies of adult flight have shown that individuals fly both up and down stream channels as well as laterally to other drainages (Kovats *et al.* 1996, Collier and Smith 1997, Turner and Williams 2000, Winterbourn and Crowe 2001). Indeed, a "colonization cycle" has been hypothesized, the central component of which is that the flight of females prior to ovipositing should be primarily directed upstream, thereby compensating for the predominately downstream movement of the immature individuals living in the water (Müller 1982). The results from field studies designed to test this colonization cycle, however, are mixed, some species clearly showing, and others not showing, directed upstream flight (Jones and Resh 1988, Hershey *et al.* 1993, Winterbourn and Crowe 2001).

Collecting adults can provide important information not always obtainable from immature forms. Species-level identifications often can be made only on adult specimens because the taxonomy of the immature forms of many groups of aquatic insects is not completely known. Collecting adults at emergence provides information critical to the understanding of the population biology, life history, and production of species as well as insight into the transfer of energy from aquatic to terrestrial food webs. Indirect measures of the extent of dispersal also have been made by determining differences in the genetic structure of subpopulations of species primarily through the sampling of adults.

Methods are provided in this chapter to (1) introduce the methodology for sampling emerging adults; (2) examine the differences in emergence that occur both spatially and temporally; (3) quantify dispersal distances of adult insects laterally from a stream; and (4) use genetic approaches to estimate the extent of dispersal in populations.

II. GENERAL DESIGN

A. Site Selection

The effective sampling of drift is best accomplished in wadeable, rocky-bottomed streams with a riffle-glide geomorphology and moderate water velocity. Drift nets become difficult to maintain or inefficient for measuring drift accurately under conditions of very high or very low water velocity. Nets can be positioned at any location within the channel, but placement in mid-stream at the downstream end of a riffle usually is most productive in terms of the number of species and individuals captured. When sampling at multiple sites located longitudinally along a channel, Koetsier *et al.* (1996) noted that drift nets should be sufficiently far apart that the organisms caught in a net will have originated from below the upstream net, thereby meeting the statistical requirement of independence of samples. This distance will vary with water velocity and other channel characteristics and is difficult to determine for any given stream; in their study, Koetsier *et al.* (1996) separated nets by at least 20 m based on the assumption that organisms typically drift <20 m during any one drift event.

Measuring the colonization of substrata and the direction of movement by the aquatic stages of invertebrates are best performed in wadeable streams that can be easily reached on a regular basis. Security from vandalism of the traps and trays frequently used in these studies also is a consideration. Streams with a gravel to cobble substratum and moderate discharge are preferable, though the general procedures work for all types of substrata. The traps and trays become clogged in streams with predominately fine-grained sediment or a high suspended solids load, affecting the colonization activity of benthic organisms.

The tent trap suggested for sampling the emergence of adult insects is best used in shallow, rocky streams. If sampling in deeper water or other types of habitats is desired, other types of emergence traps can be used (see Davies 1984).

B. General Procedures—Movements of Immature Stages

Drift. Drift is easily sampled in most streams by using drift nets set in the water for specified lengths of time (Figure 21.2). Various factors must be considered when sampling drift, including the mesh size of the net, the number of nets needed for adequate replication, sampling location, length of the sampling period, and the manner of data analysis and presentation (Brittain and Eikeland 1988).

The mesh size of the nets will depend on the objectives of the study, but the typical mesh size used is 200–300 µm. Nets with a larger mesh size will not retain small individuals that often are abundant in drift, resulting in inaccurate conclusions regarding the species composition and magnitude of drift. Nets with a smaller mesh size can be used, for example with a 40–60-µm mesh to include meiofauna in the samples, but care must be taken to ensure that the nets do not become clogged with seston.

Comparisons of the species composition, drift densities, and mean size of drifting individuals can be made between different streams or time periods (e.g., between day and night or over a 24-h cycle). The data often are quantified as *drift density*, which requires knowing the number of invertebrates captured by the nets per volume of water filtered
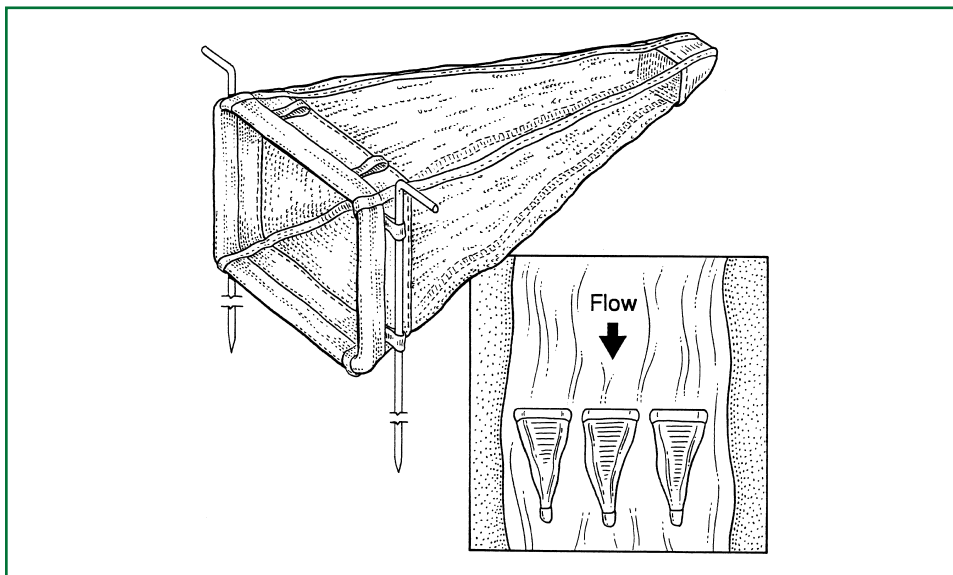
FIGURE 21.2 Drift net (modified from Merritt *et al.* 1996).

by the nets during a sampling period (Allan 1995). Drift density is usually best expressed as numbers of invertebrates drifting per 100 m³ of water:

$$Drift\ Density = [(N)(100)]/[(t)(W)(H)(V)(3600\,s/h)], \qquad (21.1)$$

where N represents number of invertebrates in a sample; t, time that the net was in the stream (h); W, net width (m); H, mean height of the water column in the net mouth (m); and V, mean water velocity at the net mouth (m/s). Faulkner and Copp (2001) present a nonlinear model for estimating the volume of water filtered by a net that corrects for the exponential decrease in V over time that occurs as seston clogs a net. Investigators requiring high accuracy in the calculation of drift density should see this paper for details on use of this approach.

Colonization Studies. Three methods are presented that examine colonization activity of lotic invertebrates. The first method focuses on colonization of a substratum by invertebrates over time. Trays filled with a substratum are placed into a stream at regular time intervals. All trays are collected at the end of the experiment and changes in the species composition and numbers of organisms colonizing the substratum over time are determined.

The second method examines the colonization by invertebrates of substrata of different sizes and thus focuses on organism-substratum preferences. Trays are filled with different-sized sediment and placed in a stream for a specified time period. The species and numbers of organisms colonizing the different sediment are then determined.

The third method examines the different routes of colonization in a stream. It follows the design of Williams and Hynes (1976) in using experimental traps placed in a stream. The traps are constructed and placed such that colonization from upstream, downstream, hyporheic, and aerial routes are separated and quantified over a given time period.

The colonization protocols require the use of trays or traps to hold substratum that serves as a colonization site for invertebrates. A wide variety of designs for trays and traps have been used for colonization studies. Two types are suggested for the methods noted above.

Colonization trays filled with clean sediment are one of the easiest methods to measure colonization. Trays are built of wood or 1.5-cm mesh galvanized-wire screening. A solid wooden frame allows use of fine-grained sediment that would not be retained by a wire mesh. A wire mesh tray, however, is preferable because the screening allows colonization to occur from all sides rather than only from the top. Dimensions of the trays typically are about 30 cm L × 30 cm W × 10 cm H.

Colonization traps, adapted from the design of Williams and Hynes (1976), measure the direction of colonization, and hence movement, in a stream. The traps consist of a basic wooden frame (50 cm L × 25 cm W × 25 cm H) with left and right sides covered with polyethylene plastic (Figure 21.3). The "upstream" and "downstream" traps also have covered tops and bottoms. Stakes placed through eye-bolts at the corners of each trap are driven into the sediment to secure the traps.

Downstream traps, which allow colonization only by invertebrates moving downstream, are open on the upstream end and have a tapered net attached to the downstream end. The end of the net is open and suspended in the water column, thereby allowing detritus and drifting invertebrates that do not colonize the substratum in the trap to pass through but inhibiting invertebrates from crawling upstream into the trap. *Upstream traps*, allowing colonization only by organisms moving upstream, are open only on the downstream end. The upstream end is covered with a 250-μm mesh netting that is protected by an outer layer of 1.5-cm mesh wire screening. *Subsurface traps*, which allow colonization only vertically from the sediment, have a solid top and a bottom covered only with wire screening. The ends of the traps are covered with wire screening and netting. *Aerial traps* have a solid bottom and upstream and downstream ends covered with wire screening and netting. Polystyrene blocks are attached to each side for flotation. Guy ropes, attached to the traps and to stakes driven into the channel, hold the traps in place while allowing them to rise and fall with the water level. A *control trap* consists of the wooden frame with a wire screening bottom and with all sides and the top open. Colonization of this trap thus can occur from all directions; it theoretically should have the highest numbers of invertebrates at the end of the colonization period.

Movements of Individual Organisms. Most of the methods noted in this chapter measure the collective movements of large numbers of individuals. Following the movement of an individual invertebrate, however, is more difficult for a variety of reasons, including the generally small size of the organisms, their frequent molting that removes external markers, and the fact that they typically dwell out of sight in the sediment. Studies need to be conducted on individuals, however, to obtain accurate measurements of the direction, frequency, and magnitude of dispersal events.

Various techniques have been used to mark individuals for use in mark-recapture studies. These include minute plastic tags, fluorescent tags, paints and dyes, abrasive
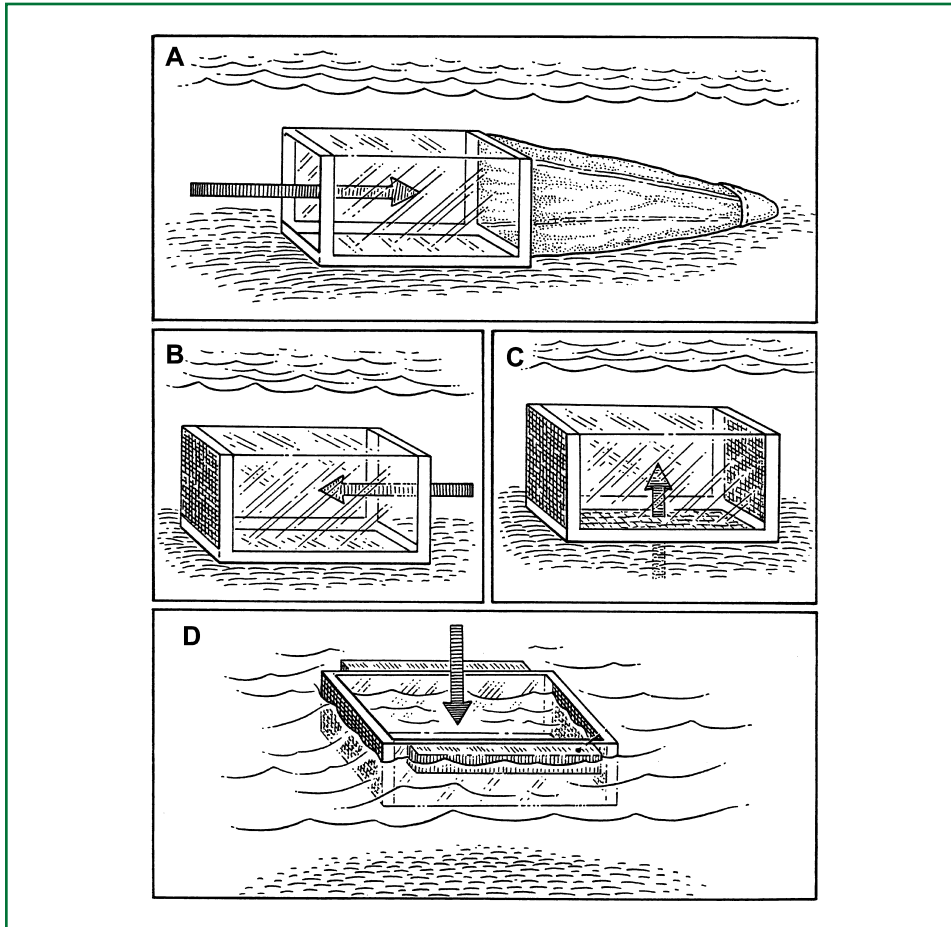
FIGURE 21.3 Colonization traps (A) Downstream movement trap, (B) upstream movement trap, (C) upward subsurface movement trap, (D) aerial colonization trap (Design modified from Williams and Hynes 1976).

markings, and radioisotopes (see Freilich 1989, Payne and Dunley 2002 for relevant references). Studies of the movements of cased caddisflies have been conducted by marking their cases, including techniques where the investigator marks the cases (see Hart and Resh 1980, Freilich 1989) and where the organisms themselves incorporate markers into their cases (Jackson *et al.* 1999). Crayfish have been marked and tracked using various methods, including using unique patterns of piercings of their uropods (Guan 1997).

Radio telemetry technology, a potentially powerful tool, is becoming more common in studies of aquatic invertebrates. This method has been employed to track crayfish (Gherardi and Barbaresi 2000, Robinson *et al.* 2000, Bubb *et al.* 2002) and larval megalopterans (Hayashi 1989, Hayashi and Nakane 1989) using transmitters affixed to the organisms. Use of radio telemetry for tracking individuals will become increasingly more feasible as transmitters continue to decrease in size and weight and increase in their signal strength. Specifics of the methods for such a study with crayfish are provided in this chapter.

C. General Procedures—Adult Emergence and Dispersal

Traps and Nets. Adult insects emerging from a stream are easily collected and quantified using emergence traps. A number of different types of traps have been developed; their designs, applicability for use under different sampling conditions, and the factors affecting their performance are discussed in detail by Davies (1984). A typical emergence trap is illustrated in Figure 21.4. The trap consists of a triangle- or pyramid-shaped wooden frame enclosing an area of 0.5–1.0 m^2 and approximately 1 m high. The sides are covered with 500-μm Nitex netting. A sample bottle, with a funnel or cone-shaped entrance to prevent insects from returning to the net, is mounted at the apex of the trap to facilitate removal of captured adults, many of which will move to the trap's top and into the bottle. The traps are placed directly on and anchored into the streambed, thereby sampling insects emerging from a known area. Traps are left in place for one day, allowing capture of insects emerging during both the day and night. In deeper water, floating traps can be employed.

Adult aquatic insects undergoing post-emergence flights can be sampled using light traps or funnel nets. Light traps are best to determine the species composition of active adults or the dispersal distances traveled laterally from the stream channel by individuals (Kovats *et al.* 1996). Funnel nets are used in studies to quantify the direction of adult flights, such as up and down stream (Turner and Williams 2000). An exercise focused on quantifying dispersal distances of adult aquatic insects laterally from a stream is provided.

Elemental and Stable Isotope Markers. More sophisticated methods of directly measuring dispersal of adults include use of elemental and stable isotope markers. Payne and Dunley (2002) marked large numbers of dragonflies with rubidium chloride (RbCl). This marker was concentrated by the larvae and then persisted in adults for up to 18 mo, allowing both short- and long-term dispersal studies to be conducted. Rb is legally
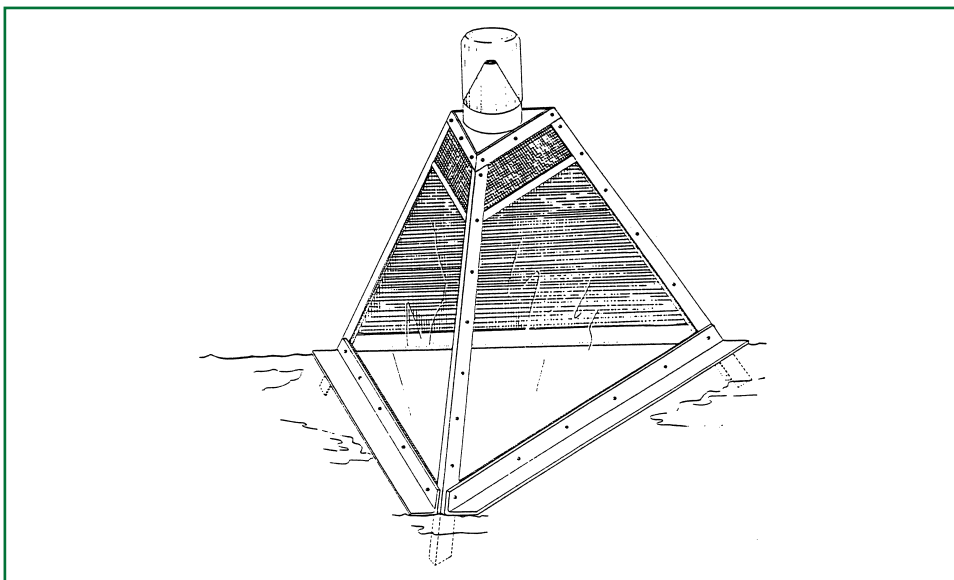
FIGURE 21.4 Mundie pyramid emergence trap (modified from Merritt *et al.* 1996).

classified as a micronutrient and thus does not require a permit for its use in the environment in most states and provinces. Detection of the Rb marker in individuals is by atomic absorption spectrophotometry.

A potentially powerful technique for measuring dispersal of aquatic invertebrates is the use of stable isotopes (see also Chapter 27). Hershey *et al.* (1993) used ^{15}N to clearly show considerable upstream flight of a significant portion of the adults of a species of mayfly. The method employs the addition of ^{15}N-enriched NH_4Cl to a stream. The ^{15}N is incorporated into epilithic microbes and algae, which in turn is ingested by benthic invertebrates that then have a different ^{15}N signal relative to nymphs elsewhere in the stream and which is maintained in the adult stage. Sampling of both pre-emergent nymphs and adults is conducted at multiple locations along the stream, including above, at, and below the enrichment site to determine the ^{15}N signal for each site. The movement of adults from the enrichment site is determined using isotopic mixing models that examine the extent of intermixing of adults from different sites (sub-populations), thereby partitioning the contribution via dispersal of different source locations of adults to a given site in the stream. See Hershey *et al.* (1993) for details on the methods and mixing models.

Population Genetics Approaches to Dispersal. Techniques grounded in population genetics have become increasingly used in the study of the dispersal of aquatic invertebrates, in particular adult insects (e.g., Hughes *et al.* 1995, Schmidt *et al.* 1995, Bunn and Hughes 1997, Monaghan *et al.* 2002, Shultheis *et al.* 2002). The basic concept is that dispersal leads to gene flow among spatially separated populations, which in turn affects the genetic structure of the populations receiving the immigrants. The extent of gene flow and the resulting differences in the genetic structure of populations thus are a measure of the extent of dispersal that has occurred between populations. High levels of dispersal between populations cause high gene flow, resulting in frequent genetic mixing and little genetic differentiation between the populations. The reverse also is true in that if two populations are relatively isolated in terms of dispersal, and hence there is little gene flow between them, then genetic differentiation between the populations is expected to be high.

A wide variety of studies can be conducted and hypotheses tested concerning the dispersal of stream-dwelling invertebrates using a population genetics approach. Most commonly the focus is on the extent of dispersal occurring between watersheds or longitudinally along a river. The geographic scale of such studies, however, can range from examining dispersal between neighboring low-order streams to dispersal between geographically distant watersheds. This approach also can be used to determine the effects of geographic isolation barriers on dispersal of aquatic organisms and to determine the source population of immigrants to a given site.

III. SPECIFIC METHODS

A. Basic Method 1: Quantifying Invertebrate Drift

Objective: Compare the drift density of invertebrates in a stream between day and night.

1. Place drift nets across the stream channel (Figure 21.2). Nets are placed in the stream with the net face perpendicular to the direction of flow and anchored with rods driven into the substratum. Nets should be positioned at mid-depth in the

water column or, if the stream is shallow, the bottoms of the nets should be 2–3 cm above the sediment to reduce the possibility of invertebrates crawling into the nets. Presuming channel width permits, use at least three nets positioned side-by-side (on a transect across the stream) to provide replicate measurements.

2. Calculate the volume of water passing through each drift net by determining the area of the water column being sampled and the average water velocity at the mouth of each net. Record the width of the net mouth and then measure the depth of water entering the net at a minimum of three equidistant locations across the mouth. Use a flow meter to measure water velocity at the three locations (see Chapter 3).

3. Record the time the nets are placed in and removed from the stream. Generally strive to keep the nets in the stream for 1-h periods. This period may have to be shortened if high seston concentrations cause the net mesh to clog, thereby reducing flow.

4. Remove the nets from the stream and wash the contents into a bucket partly filled with water. Use forceps to remove any invertebrates that remain clinging to the inside of the nets. Wash the contents of the bucket through a sieve with a mesh size equal to or smaller than that of the net. Preserve the material from each net separately in bottles or sealable bags with 70% ethanol (final concentration). Label the samples with location, date of collection, net number, time period of sampling, and investigator.

5. Repeat steps 1–4 for a minimum of three consecutive 1-h periods both before and after sunset. If possible, sample over an entire 24-h period. Nets should be placed in the same location during each sampling interval.

6. In the laboratory, separate all organisms from the debris in the samples. This is best accomplished using a stereomicroscope at low power. Count the number of invertebrates in each sample. Record data on Table 21.1.

7. Calculate the mean drift density of invertebrates in the stream during each time interval. Construct a curve showing the change in drift density over time (e.g., Figure 21.1). Use a *t*-test or nonparametric Mann-Whitney *U*-test to test the null hypothesis that there was no significant difference between day and night drift densities. If samples were taken over a series of consecutive time periods such as over 24 h, and thus may not be independent of each other, use time series analysis to examine diel drift patterns (e.g., Schreiber 1995).

B. Basic Method 2: Species and Size Composition of Drifting Invertebrates

Objective: Using the samples collected in Basic Method 1, determine if differences exist in the species composition or mean size of invertebrates drifting at different times of the day.

1. To determine species composition, identify and enumerate the taxa of invertebrates in the samples (see Chapters 20 and 25). Determine if there was a change in the species composition and relative abundance of drifting organisms during the different time periods sampled using a similarity index or ordination technique such as detrended correspondence analysis (e.g., Schreiber 1995).

2. Determine the size of each individual collected in the samples. This can be accomplished by various methods. (a) Place all invertebrates collected in a sample together in an aluminum weighing pan and dry them in a drying oven for 24 h at 60°C. Weigh the pooled invertebrates on an electronic balance. Calculate the mean

| TABLE 21.1 | Sample Data Sheet for Benthic Invertebrate Drift. |
| | |

Stream name: Date:

Stream location: Investigator:

Net mesh size: Net mouth width:

	Net 1	Net 2	Net 3
Time net put in			
Time net taken out			
Total sampling time (hr)			
Water depth at net (cm) 　　Point A 　　Point B 　　Point C			
Water velocity at net (m/s) 　　Point A 　　Point B 　　Point C			
Number of macroinvertebrates			
Mass of macroinvertebrates (mg)			
Species composition 　　Taxon A 　　Taxon B 　　Taxon C 　　Etc.			

individual dry mass of the drifting organisms (= pooled dry mass/number of individuals in the sample). (b) Measure the length of all invertebrates in each sample using an ocular micrometer fitted in a stereo-microscope. Calculate the mean length of the organisms in each sample. (c) If desired, the mass of each individual can be estimated using the measured lengths and published regression equations relating organism length to mass for a wide variety of aquatic invertebrates (Benke *et al.* 1999).

3. Construct a figure showing the mean size or mass of invertebrates drifting during each time period. Use a *t*-test or Mann-Whitney *U*-test (Zar 1999) to test the null hypothesis that there was no significant difference between the mean size or mass of invertebrates drifting during the day and night.

C. Basic Method 3: Colonization of Substrata over Time

Objective: Determine the changes in the species and numbers of invertebrates colonizing substrata over time.

1. Fill a minimum of 15 colonization trays with sediment of a uniform size that is similar to that of the predominant particles in the stream. Fifteen trays provide

three replicate trays per time period. Additional trays can be used to increase the number of replicates per time period or to increase the number of time periods that trays are collected.

2. Place three replicate trays into the stream on each of five days spaced over two to three weeks (e.g., at four-day intervals). Trays should be buried such that their tops are flush with the stream bed.

3. Retrieve all trays one to two days after the last set of trays are placed into the stream. Thus, the *simultaneous-removal* method for determining colonization is used for this exercise. Trays are placed in the stream periodically over the entire colonization period. All trays are retrieved together at the end of the study, thereby subjecting all substrata to the same potential colonizing species and to the same environmental conditions at the end of the colonization period (Shaw and Minshall 1980).

4. Wash invertebrates associated with the sediment in a tray into a bucket. Pass the contents of the bucket through a 250-μm mesh sieve and preserve and label the material retained by the sieve. Add a small amount of Rose Bengal dye to the sample to aid in the separation of organisms from debris.

5. In the laboratory, remove, identify, and count all invertebrates in each sample. Record data in Table 21.2.

6. Calculate the mean number of taxa and mean number of individuals that colonized the sediment during each time period. Construct colonization curves illustrating changes in numbers of individuals and taxa over time (e.g., Figure 21.5). Use an ANOVA or Kruskal-Wallis test (Zar 1999) to test the null hypothesis that no significant difference existed in the number of taxa or individuals colonizing the substratum over time. Use a multiple comparison test (e.g., Scheffe or Tukey procedures) to determine the number of days it took until there was no significant increase in the number of taxa or individuals that had colonized the substratum.

TABLE 21.2 Sample Date Sheet for Benthic Invertebrate Colonization Over Time.

Stream name:		Date:		
Stream location:		Investigator:		
Tray number				
	1	2	3	4
Day tray put in				
Day tray retrieved				
Total days in stream				
Number of macroinvertebrates				
Mass of macroinvertebrates (mg)				

Species composition
    Taxon A
    Taxon B
    Taxon C
    Etc.				
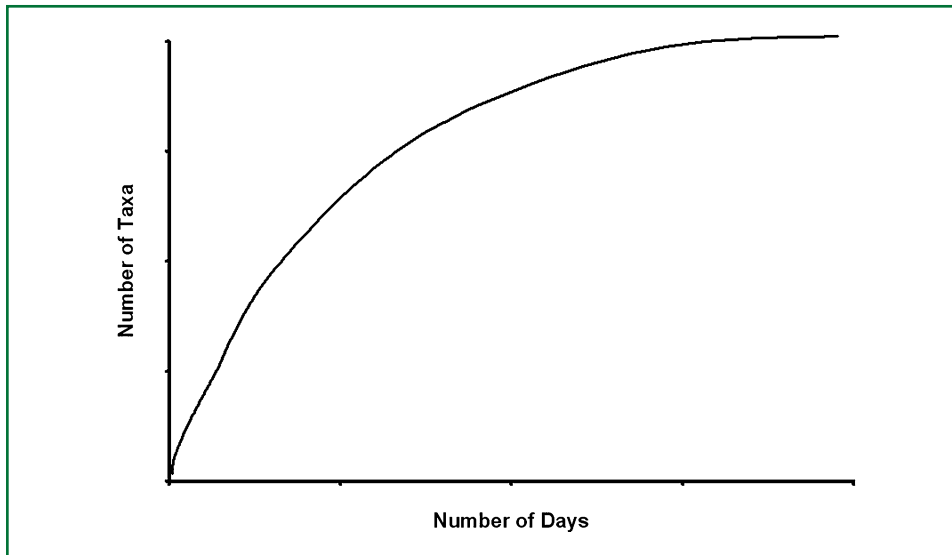
FIGURE 21.5 Sample colonization curve showing changes in number of taxa colonizing a substratum over time.

7. *Optional*: Dry (60°C for 24 h) and weigh the invertebrates to determine changes in biomass of the organisms colonizing the substratum over time. Use regression analysis (Zar 1999) to determine if there was a trend in the mean size of colonizing individuals over time (mean size is the biomass in a sample divided by the number of organisms in the sample).

D. Basic Method 4: Effect of Substratum Size on Colonization

Objective: Determine the effect of sediment particle size on the colonization of substrata by invertebrates.

1. Collect sediment and separate by sieving into four size categories. The suggested range in particle diameter sizes is from about 0.2 to 63 mm. Potential size categories are 0.2–2, 2–16, 16–37, and 37–63 mm. Wash and dry the sediment to remove all invertebrates.
2. Place the sediments into a minimum of 12 colonization trays, three each for each of the size categories. Use additional trays if possible to increase replication. Bury the trays in the stream with the tops flush with the stream bed. Locate the trays such that water velocity is similar across their tops.
3. Retrieve the trays after 7–14 days. Preserve samples with 70% ethanol, label, and add Rose Bengal.
4. In the laboratory, remove, identify, and count all invertebrates in each sample.
5. Calculate the mean number of taxa and mean number (and optionally biomass) of individuals that colonized each particle size. Use an ANOVA or Kruskal-Wallis test to test the null hypothesis that there was no significant difference in the number of taxa or individuals colonizing the different particle sizes. Use a

multiple comparison test (e.g., Scheffe or Tukey procedures) to identify significant differences among the means.

6. *Optional*: A more extensive study is to combine the procedures of Basic Methods 3 and 4, thereby examining if significant differences occur in the rate of colonization of different sediment particle sizes.

E. Advanced Method 1: Mechanisms of Colonization by Invertebrates

Objective: Determine the relative importance of different mechanisms of colonization of substrata by lotic invertebrates.

1. Wash and dry sediment from the stream to remove all invertebrates.
2. Fill colonization traps with the sediment to a depth of 5 cm. Use a minimum of three downstream, upstream, subsurface, and control traps; aerial traps can be included if this route of colonization is to be included in the study. Bury the traps such that the top of the sediment in each trap is flush with the streambed. The aerial traps are not buried, but rather are allowed to float at the water's surface. Placement of the traps should be such that water velocity at all traps is similar.
3. Leave the traps in place for a minimum of 7 days. A longer period of up to 30 days, however, is preferable to ensure complete colonization of the substratum. A minimum of three weeks usually is necessary if aerial traps are used. The netting must be periodically cleaned to allow free flow of water through the traps.
4. At the end of the colonization period, raise the traps slightly and slip a 250-μm mesh bag around them to prevent loss of animals before lifting the traps from the streambed. Preserve the material in each trap with 70% ethanol, label the samples, and add Rose Bengal.
5. In the laboratory, remove, identify, and count all invertebrates in each sample. Record the data in Table 21.3.
6. Calculate the mean number of taxa and number of individuals that colonized each set of traps. Use an ANOVA or Kruskal-Wallis test to test the null hypothesis that there was no significant difference in the number of taxa or individuals colonizing by the different mechanisms. Use a multiple comparison test (e.g., Scheffe or Tukey procedures) to partition significant differences among the means.

F. Advanced Method 2: Activity Patterns of Crayfish Using Radio Telemetry

Objective: Determine the diel activity pattern of crayfish in a stream.

1. Capture crayfish from a stream using a seine, dip net, crayfish trap, or by turning over stones while snorkeling and collecting them by hand. Place crayfish in an aerated cooler and return to the lab.
2. A variety of telemetry equipment is commercially available. Instructions for use of the transmitter, antenna, and receiver are specific to the product. Important specifications to consider include that the weight of the transmitter should be less than 10% of the body weight of the crayfish, that the system be such that the signal strength can be detected at a minimum of 25 m, that the transmitter's location can be determined within 0.3 m, and that its battery life is appropriate to the length of the

TABLE 21.3 Sample Data Sheet for Mechanisms of Colonization by Benthic Invertebrates.

	Number of taxa	Number of individuals
Stream name:		
Stream location: Investigator:		
Date traps in: Date traps out:		
Downstream trap		
Trap A		
Trap B		
Trap C		
Upstream trap		
Trap A		
Trap B		
Trap C		
Subsurface trap		
Trap A		
Trap B		
Trap C		
Aerial trap		
Trap A		
Trap B		
Trap C		
Control trap		
Trap A		
Trap B		
Trap C		

    study. Use multiple crayfish and transmitters, with a minimum frequency spacing of 10 kHz, to identify individual crayfish, for simultaneous replication of the study.

3. Attach a transmitter to the chela of a crayfish using the technique of Robinson *et al.* (2000) or Bubb *et al.* (2002). Bubb *et al.* (2002) note that affixing the transmitter to the chela causes less inhibition of movement than if it is placed on the celphalothorax. After drying the chela, use an acrylate adhesive to attach the antenna and then use denture cream, available at drug stores, to fill in the crevasses between the chela and antenna. Place the crayfish on a damp cloth in a cooler while letting the adhesives dry for 30 min before placing the organism in water.

4. Place the crayfish in the stream with a bottomless cloth mesh cage over the organism. The cage will insure that the crayfish settles safely into the substratum and allows the investigator to determine that the transmitter is operating properly. Record the starting location of the organism using a GPS system and, after a minimum of 30 min, remove the cage.

5. Diel activity: Determine the location of the crayfish at standard time intervals over 24 h to track movement over a full day-night cycle. Time intervals should be a minimum of 1 hour; use shorter intervals if greater detail on activity patterns is desired. Use a GPS system to record the location of the crayfish. Data analysis may include the following: (1) Determine the total distance moved during a 24-h cycle.

This equals the sum of the distances moved during each time period. (2) Determine the net distance and direction moved. This is the distance between the initial and final location and the upstream or downstream vector angle from the initial location. (3) Compare the total distance moved and the mean distances moved during each time period during day and night periods to determine if there was a difference in day versus night movement activity.

6. Many modifications of the objectives of this study are possible, such as examining activity patterns during different flow regimes or seasons or under differing levels of potential predation pressure or competition.

G. Basic Method 5: Emergence of Adult Aquatic Insects

Objective: Determine if differences exist in the numbers and species of adults that emerge from different habitats within a stream. Optionally, determine if the numbers and species emerging differ between day and night.

1. Anchor emergence traps over the primary habitats in a stream. Typical habitats include riffles, pools, glides, or debris accumulations. Record the characteristics that differentiate the habitats (e.g., particle size or type, water velocity, water depth).
2. After 24 hr, remove all insects from the sample bottle and netting and preserve them with 70% ethanol. Label the sample with location, trap number, date, sampling period, and investigator.
3. *Optional*: Rather than sampling different habitats, divide sampling into day and night periods, recording the number of hours the traps were in place during both periods.
4. In the laboratory, identify and count the number of insects collected in each trap. Record data in Table 21.4.
5. Express the results as the mean number of taxa or individuals emerging per square meter per hour from each habitat or during the day and night. Use a *t*-test or ANOVA (or corresponding non-parametric test) to test the null hypothesis that there was no significant difference in the number of taxa or individuals emerging from the different habitats or during the day and night.

H. Basic Method 6: Inland Dispersal of Adult Aquatic Insects

Objective: Quantify the extent to which adult aquatic insects disperse laterally from a stream.

1. Establish sampling locations at a minimum of five locations, such as 0, 25, 50, 100, and 200 m on a transect away from the stream. Other spacing regimes may be used depending on the size of the drainage basin and its geography.
2. Set up light traps at each sampling location. Commercially purchased light traps may be used, typically with 40–50-cm 12 V/15 W DC fluorescent long-wave ultraviolet lamps powered by two 6V dry cell batteries. Traps usually are designed such that the lamps are situated vertically among three plastic vanes and located over a funnel that serves as a lid to a bottom bucket. The bucket, sitting on a broad white sheet, should contain dry ice in a wire mesh basket. Insects that are attracted to the light and strike the vanes fall through the funnel and are frozen by the dry ice. Insects that land on the sheet can be collected with forceps.

TABLE 21.4	Sample Data Sheet for Adult Insect Emergence.

Stream name: Date:

Stream location: Investigator:

Type of trap: Trap sampling area:

Habitat sampled	Number of taxa	Number of individuals

Habitat 1
    Trap A
    Trap B
    Trap C		

Habitat 2
    Trap A
    Trap B
    Trap C		

Habitat 3
    Trap A
    Trap B
    Trap C		

TABLE 21.5	Sample Data Sheet for Lateral Dispersal of Adult Insects.

Stream name: Date:

Stream location: Investigator:

Weather conditions:

Distance from stream channel _____

Time light trapping initiated _____ Time ended _____ Total time elapsed _____

Taxon	Number of individuals
Taxon A	
Taxon B	
Taxon C	
Etc.	

3. All light traps are operated simultaneously for 2 hr immediately after sunset. Preserve the samples in 70% ethanol. Label the samples as above.
4. In the laboratory, identify and count the number of insects of aquatic taxa collected in each trap. Record data in Table 21.5.
5. Express the results as the number of taxa or individuals captured at each trap. If traps were operated for different time periods, express the results per a standard time period. Develop a graph showing the change in number of taxa or individuals with distance from the stream.

I. Advanced Method 3: Dispersal of Adult Aquatic Insects Using a Population Genetics Approach

Objective: Determine the extent of dispersal between populations of an aquatic invertebrate by measuring the extent of gene flow between the populations.

1. The assessment of genetic differentiation is based on how the spatial distribution and relative frequencies of alleles at polymorphic loci differ between populations. A variety of techniques have been employed for this purpose, including analysis of allozymes, mitochondrial DNA, randomly amplified polymorphic DNA, and microsatellite loci (Bilton *et al.* 2001). It is best if an approach that uses several independent molecular characters derived from among these techniques is incorporated into the study design.

2. Various statistical approaches are available to quantify gene flow from data produced by these analytical techniques (Bilton *et al.* 2001). Among the more commonly used statistics in studies of aquatic insect dispersal are Wright's F_{st}, Weir's θ, and nested clade analysis. F_{st} measures the proportion of total variation that occurs between populations, varying from zero, when the same allele is shared in equal frequencies by all populations, to one, when fixed allele differences occur between populations. Weir's θ is a measure of the relative fixation of alternate alleles in different subpopulations. It thus is an estimate of co-ancestry, or the degree of relationship between genes of different individuals in one or more populations. Nested clade analysis, a type of phylogeographic analysis that estimates temporal-spatial genetic variation, is better suited than more traditional approaches for differentiating historical versus ongoing dispersal and detecting geographical associations among populations. Multiple assumptions, including those concerning the constancy of population size and dispersal patterns and the effect of disturbance on the ability of populations in streams to achieve equilibrium levels of genetic divergence, are inherent in these statistics and should be recognized by the investigator.

3. The specific sampling, laboratory, and statistical techniques used to determine genetic differentiation vary greatly depending on the methods employed and are beyond the scope of this book. The studies of Bunn and Hughes (1997), Monaghan *et al.* (2002), and Shultheis *et al.* (2002), and references in Bilton *et al.* (2001) can be used as a guide for appropriate procedures to be used for these studies.

IV. QUESTIONS

1. Are the species collected by the drift sampler representative of the entire benthic invertebrate community in the stream?

2. What differences might occur in drift densities and the species drifting as stream size (order) increases? Would you expect seasonal differences in drift densities? Why?

3. What differences might occur in the drift densities of different-sized (e.g., large vs. small) invertebrates in streams with and without fish? Might the presence of fish have a different effect on day versus night drift densities compared to in a fishless stream?

4. Large numbers of invertebrates can be transported downstream by drift each day. Why don't the upstream reaches of streams become depleted of invertebrates?

5. Did the colonization curves reach an asymptote? If not, what does this suggest about the colonization process for your substratum?

6. What are some of the characteristics of the substrata used in your colonization study that may have been responsible for the differences in the numbers and species of invertebrates that colonized each type of substratum?

7. To what extent was the downstream movement of invertebrates compensated for by upstream movements? By movement up from the subsurface sediments or from aerial colonization?

8. What are the primary factors that might affect the frequency and extent of movements of an individual invertebrate?

9. What factors might be responsible for differences in the numbers of emerging insects among different substrata? What factors might cause day-to-day variation in the numbers of emerging insects?

10. Benthic sampling devices (e.g., Chapter 20) and emergence traps often provide very different estimates of the species composition of stream invertebrate communities. What are some of the reasons for these differences?

11. Do the numbers of adult taxa collected laterally from the channel show an exponential decrease with distance from the channel? Why or why not?

12. Would you expect greater genetic differentiation when comparing populations of a species of aquatic insect that occur in closely neighboring headwater streams from different river systems or when comparing populations of a species that occurs in the headwaters and high-order sections of one river system? What factors affect the extent of genetic differentiation observed under these two scenarios?

V. MATERIALS AND SUPPLIES

Field Materials

Drift nets
Colonization trays
Colonization traps
Emergence traps
Current velocity meter
Meter sticks
Buckets (5 gallon)
Sieves (series of mesh sizes)
Forceps
Ethanol (70%)
Bottles or sealable bags
Labeling paper and pencils
Hip boots or waders
Telemetry equipment—transmitter, antenna, receiver
Global positioning system unit
Light traps
Coolers

Laboratory Materials

Stereomicroscopes
Ocular micrometer (optional)
Drying oven (optional)
Electronic balance (optional)
Rose Bengal
Aluminum weighing pans
Acrylate adhesive

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Role of Fish Assemblages in Stream Communities

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I. INTRODUCTION

Community structure can be measured in three ways: structure, function, and the combination of structure and function. The first aspect of structure is the listing of community elements. This can be simply a species list, but also could be expressed as the number and relative composition of community components. In this form, linkages among species or community elements are unspecified. The species list is a description using Linnaean taxonomy; which, in and of itself, provides very little ecological information. However, species can be grouped in terms of ecological function, as guilds or functional groups.

Guilds are groups of species that use the environment a similar way. For example, there are coldwater and warmwater guilds, riffle guilds and pool dwelling guilds, and other possible classifications. Curiously, although guild members need not be closely related, guild descriptions from past studies have been from closely related taxonomic groups (i.e., taxocenes). One problem with describing guilds by taxocenes is that the niche of fishes changes dramatically through development (ontogeny). For example, the largescale sucker (*Catostomus macrocheilus*) begins life by feeding on tiny invertebrates; as the mouth metamorphoses from the terminal position to the subterminal position it shifts to larger benthic invertebrates, then to a mixture of invertebrates, detritus and diatoms. Finally, the diet of the large adults is mostly diatoms (D. H. Bennett, personal

communication). To which guild do largescale suckers belong? If described by ecological processes, they would belong to several groups. Although it is not impossible to devise a system in which a species could belong to multiple guilds, a system of functional groups explicitly assumes that a species could belong to several groups.

Functional groups are defined as species aggregations functioning in the community in a similar way. Fish change ecological roles at different life stages. During their life span they can operate as different ecological species and could be grouped with various taxocenes over time. For example, the adult largescale sucker might be lumped together with snails and caddisflies as scrapers, while the juveniles can be categorized with predacious stoneflies as benthic invertivores (see Chapter 25). Unlike guilds, functional group classifications often combine several taxocenes.

The second phase of understanding community structure is to delineate linkages among community members. As a metaphor, it is difficult to understand how a disarticulated skeleton functions if you don't know that "the foot bone is connected to the ankle bone." The same applies to understanding communities. A *community* is a self-sustaining system through time and space, although the relative composition of its elements may vary through time at any point in space. It is composed of primary producers, decomposers, herbivores, detritivores, omnivores, and carnivores. Fishes occur at multiple trophic levels but constitute only an *assemblage* within the larger community. By themselves, they cannot sustain the system.

An ecological study of fish assemblages is a search for pattern. The methods in this chapter provide tools for conducting a comparative study of fish assemblages by (1) recognizing and measuring differences in physical habitats; (2) collecting, identifying, and enumerating different types of fishes; (3) determining the degree of similarity among the fauna at different sites; (4) relating habitat characteristics to the abundance and distribution of particular fishes; (5) comparing species memberships and morphological diversity among species; and (6) understanding the fish's role in community structure through graphical techniques. We present and discuss the assumptions and uses of various species indices, and also briefly discuss modern ordination techniques. The topics in this chapter could easily fill several books. We recommend the following readings for those interested in further inquiry: stream fish ecology (Bayley and Li 1992, Matthews 1998), use of indicators for monitoring purposes (Karr *et al.* 1986, Kwak and Peterson in press), community analysis (McCune and Grace 2002), sign digraphs and qualitative mathematical analysis (Levins 1973, Bodini *et al.* 1994, 2000, Hulot *et al.* 2000, Dambacher *et al.* 2002a, 2003a, b).

II. GENERAL DESIGN

The study of an entire community is extremely challenging. Such studies are best done by teams because they require an examination of how physical and biological components of a system interact. Complexity forces the scope of study to be reduced to fit the constraints of time and resources. Virtually all studies of communities are incomplete, but decisions are made to optimize more limited objectives. We discuss how to study one taxocene — fish — in relation to community processes. Various aspects of the methods herein require that you have completed a variety of basic work from other chapters of this book, especially stream physical measurements (see Section 1), before undertaking the approaches in these methods. Sites geo-referenced on maps, data collection and management, and specimen maintenance and storage should be coordinated so that

combining information will be compatible and therefore comprehensible. Answers to the following questions will help to modify priorities and allocation of effort for your study.

Questions to consider while planning a study of fish communities:

1. What is the nature of the community? How is composition of the fish community within the ecosystem to be defined? Where do the various bio-components of the ecosystem interface? What is the best way to describe the community components — by species — filamentous algae? diatoms? bacteria? fungi? aquatic invertebrates? riparian organisms? terrestrial invertebrates? fishes? birds? mammals? amphibians? or by functional groups?
2. What temporal extent is sufficient to address community process of interest — one season, every season? Is interannual variation important? Are multiple years required as in a study of community responses to recurrent disturbances and disturbance frequencies?
3. Where in the basin is the community located? What is the spatial scale needed to observe the community process in question — channel unit? stream reach? valley type? subbasin? basin?
4. What relevant and reliable previous information exists?

Prior information may exist if the research team or class develops a database over time. Alternatively, published faunal guides or stream ecologists in your region can be rich sources for natural history information. Also, Long-Term Ecological Research sites sponsored by the National Science Foundation encourage comparative studies and their large accessible databases may accommodate your interests (see http://www.lternet.edu/sites/).

General steps to consider in describing fishes of an aquatic assemblage:

1. Capture organisms and document characteristics of the location and habitats in which they were found.
2. Identify organisms by taxon and function.
3. Quantify captured organisms by numbers and weight. Classify this information by taxon and functional groups.
4. Identify interactions (links) among the variables (biological species, ecological species, functional groups) and create a community web.
5. Quantify the interaction strengths (this generally requires experimentation).
6. Shift the emphasis from static description of habitat to understanding dynamic patterns (this generally requires both experimentation and quantitative modeling).

In this chapter we will cover all steps except for 5 and 6, which are particularly advanced and would require multiple sessions.

A. Site Selection

A critical consideration is the spatial and temporal extent over which samples should be taken. It should be large enough to encapsulate the life history requirements for the species in question (Fausch *et al.* 2002) and, ideally, sampled during all seasons because of shifting habitat usage. However, these criteria are often not known and may be the objective of the study. If these considerations are not possible, we must acknowledge that the boundaries of the system of study have been arbitrarily determined. This becomes an

issue when the community under question is found to be unstable using mathematical criteria. This may be an artifact of scale and not of the assemblage structure *per se*.

It is important to consider stream habitats as being hierarchically nested across multiple spatial scales (see Chapters 1 and 2). When habitats are classified, enumerated, and spatially referenced, a nested, stratified-random sampling protocol can be used to identify similarities and differences among sites in some instances. This classification places the sampled fish assemblages in context with their environment and helps to ensure that sites are comparable for the contrasts of interest. Certain types of habitat specialties can be detected more easily. Some fishes live in higher gradient reaches of the river system (i.e., headwater habitats, or "rhithron") whereas other species are only found in stream reaches of the lower gradient, wide alluvial valleys ("potamon"; Bayley and Li 1992). For example, in the Cascade Mountains of Oregon, cutthroat trout (*Oncorhynchus* clarki) are the only fish found in stream reaches (riffles and pools) with gradients of 15%, but coho salmon (*Oncorhynchus kisutch*) are found mostly in pools in stream reaches where gradients seldom exceed 4–5%.

In addition to site-specific habitat measurements, observations should be made of (1) position of the sites within the watershed; (2) watershed conditions upstream and down (e.g., land-use patterns, barriers to dispersal, potential for cumulative influences); and (3) factors associated with changes in stream power (e.g., discharge, gradient, incoming tributaries).

B. Habitat Inventory

To organize the data as a nested spatial hierarchy, place habitat and fish inventories in order of habitat size, where basin > subbasin > stream > valley segment > reach > channel unit > microhabitat (Frissel *et al.* 1986, Poole *et al.* 1997). This inequality indicates that smaller habitats are nested within larger categories. There are at least three reasons for this organizing strategy. First, as mentioned above, strata can be identified for a nested sampling design. Second, certain habitat properties emerge at particular hierarchical levels. Depending on life history stage, fish respond differently to varying geophysical and biophysical processes, often characteristic of specific parts of a watershed (Montgomery 1999; see also Chapter 2). Third, this hierarchy provides the context for understanding associations between assemblage structure and habitat, possibly at multiple levels of spatial organization (e.g., by valley type, stream segment type, and habitat unit type). Not surprisingly, differences exist in the array of *habitats units* available among valley types, and it is reasonable to assume that different types of organisms will be found among them. At the *channel unit* level, this becomes more clear. Certain types of fishes inhabit pools (e.g., juvenile chinook salmon *O. tschawytscha* and redside shiners *Richardsonius balteatus*) whereas other taxa are found in faster water (e.g., bonytails *Gila elegans* and riffle sculpins *Cottus gulosus*). Environmental gradients of volume, discharge, elevation, trophic productivity, temperature, dissolved oxygen and pH that follow the River Continuum (Vannote *et al.* 1980) clearly impose patterns on fish distributions, but discontinuities still appear that may be explained by geomorphic anomalies. Certain valley segments or stream segments may have distinct faunal signatures because of the types of habitats they support or the presence of distinctive features. For example, the Deschutes River in Oregon has several geological features that differ from a "typical" river system. From the mountainous headwaters to a series of lowland falls called Steelhead Falls, you see the typical transition from coldwater to warmwater fishes. Below the falls, however, massive coldwater springs create a sudden shift back to a coldwater fish fauna.

C. Preparatory Work

Part of the inventory should be done in preparation for fieldwork as follows:

1. Using high-resolution and high-quality maps, and aerial photographs if available, decompose the watershed into a spatial hierarchy (see Chapters 1 and 2).
2. If the basin is sufficiently small, a walk through the river valley with a GIS unit and a map will suffice (alternatively map, compass, and pedometer).
3. Photo points of the boundaries of each nested spatial category will be useful.
4. In the laboratory, identify homogenous geomorphic units at different spatial scales. A geomorphic unit can be characterized by its subunits. For example, plunge pools are characteristic streams flowing through narrow, steep, valley segments. In contrast, side-scour pools, those which are formed on the outside of a meander bend, are strong features of streams meandering through a low gradient, alluvial valley. The fauna found in each valley type will be distinctive. If you lump all pools without regard for valley type, you will be lumping apples with oranges, so to speak. Therefore each valley type will form a sampling stratum. If the basin is small, geomorphic differences may be small and identification of strata may be unimportant.

The length of the stream to be sampled depends upon stream size and the scale of interest, but also upon regional characteristics. As an illustration, Reynolds *et al.* (2003) determined that, on average, a stream length equaling 40 channel wetted-widths was adequate to characterize 90% of the fish fauna in wadeable streams of the Willamette Valley, Oregon. Forty channels widths may not work in all instances but may represent a reasonable starting point. You may choose to sample representative channel units (e.g., pools and riffles) within valley segments rather than the entire stream, but the rough rule-of-thumb of 40 stream widths may be useful to determine the sampling boundaries.

Prior to fish sampling, habitat sampling should be conducted in the field as follows:

1. Conduct a hydrologic assessment of your sampling sites including measures of area, volume, discharge and habitat hydraulics (see Chapters 3 and 4).
2. Collect other physical data for factors known to be important to fish abundance and distribution such as water temperature, canopy cover, instream cover, dissolved oxygen, hyporheic upwelling, and turbidity (see Chapters 5, 6, and 7). Other variables may be equally or more important than those listed (e.g., pesticide runoff, industrial discharge) and may require measurement but first check with the appropriate municipal, county, state or provincial, and federal agencies that may have the information you seek.

D. General Approaches

For each of the methods we suggest a comparative approach using minimally two to three sites. If there is no burning hypothesis that you wish to test, we suggest you consider examining the distribution and abundance of species along the stream profile (i.e., along the River Continuum); this is a time-honored type of study and one that is very instructive (Huet 1959, Sheldon 1968, Horwitz 1978, Rahel and Hubert 1991). If you are time-limited, compare habitats from two very different parts of the watershed basin. The former can be an exercise for an entire term; the latter is a two-week project. You

have already examined the geographic and cartographic information and devised your sampling plan. The methods in this chapter will provide sequentially more information as you compare sites.

Several methods can be used to sample fishes. If fish are well known and numbers of species are limited, *visual survey* by snorkeling divers can be employed in streams that are highly transparent, especially when water is waist-deep or higher. Snorkeling is also the best technique to census small juvenile fishes. However, this technique is biased against detection of fishes that live in the interstitial spaces within the substrates (e.g., sculpins, darters). Snorkeling may not be possible in all streams because of turbidity or insufficient depth (see Helfman 1983 for snorkeling methods). Two other common methods to sample fishes are *electrofishing* and *seining*. Electrofishing is an excellent tool when the habitat has many snags that can foul a net, but fish escape capture when water is thigh-deep or higher. Also, electrofishing is biased for large fish. Check with management agencies for local regulations. Some areas require training and certification before electrofishing will be allowed. Seines are excellent for large streams with deep channels, slow current, and sandy, muddy, or pebbly substrates. Seines with small mesh nets (0.5-cm stretch) capture small fish efficiently, but they are less efficient in channels with high roughness (e.g., numerous snags or boulders). Some investigators combine both techniques by using the electrical pulse to chase fishes into seines positioned downstream, which maximizes the strengths of both fishing methods. Regardless of gear, accurate assessments of assemblage structure require good estimates of population abundances of *all species.*

We present three methods for estimating population sizes: (1) visual estimation; (2) mark-and-recapture techniques; and (3) the depletion method. Methods (1) and (3) generally require only a single visit to the study site. The Peterson or single-census mark-and-recapture technique requires two sampling efforts. The first effort captures fish to mark, after which they are released. Fish captured on the second sample determine the population estimate. This method assumes that the ratio of released marked fish (M) to the total population (N) is the same as the ratio of marked fish recaptured (m) to the total fish captured (n), comprising marked and unmarked individuals, on the second sample (Ricker 1975, Caughley 1977):

$$M/N = m/n \qquad (22.1)$$

Other assumptions are that marked fish will be randomly distributed within the entire targeted population, that each fish has equal probability of capture, and that the population is closed.

When studying fish communities, there is a legitimate need for preserved specimens constituting a reference collection. These specimens are needed as vouchers to assure quality control and quality assurance on identifications. Persons first learning the fauna often cannot distinguish certain key characters without substantial handling and even dissection (e.g., pharyngeal tooth counts, pyloric caeca counts, lateral line counts, color of the peritoneum). Morphology provides clues to fish ecology (e.g., Keast and Webb 1966). For example, feeding efficiencies are related to not only the position and relative size of the mouth (mouth gape) but also the anatomy and biomechanics of the upper jaw and expansion of the bucchal cavity as a coordinated unit. Careful dissection is a critical tool for understanding. For a collection caught at a particular place and time,

specimens form a historical record of the assemblage. Future scientists can visit the collection and use the specimens for other purposes. However, the life of these organisms should be respected and whenever possible fishes should be returned to the stream. As an illustration, fish collected for diet analysis can be studied using nonlethal gastric lavage (stomach pumping) instead of killing the fish and dissecting the gastrointestinal tract (see Chapter 26).

Indices of Community Structure and Function. Indicator species can be associated with certain types of habitats and results from studies may identify an indicator. In Europe, Huet (1959) described species associations found common to particular stream environments but not found elsewhere (i.e., trout, grayling, barbell, and bream zones). Certain species have stringent requirements. For example, rainbow trout (*O. mykiss*) are sensitive to low pH water that inhibits reproductive success; their absence may be an early warning of acidified waters. Other species may be found across a broad range of conditions. For example, red shiners (*Cyprinella lutrensis*) and speckled dace (*Rhinichthys osculus)* are eurytopic (i.e., habitat generalists). Both fishes are associated with other species under benign conditions, but are often the only species found under the harsh conditions in desiccating pools during summer base flow. These fishes can be clear indicators of severe conditions. Some fishes are "umbrella" species whose presence is highly correlated with the other species within the assemblage. Stream-dwelling bull trout (*Salvelinus confluentus*) are stenotopic (i.e., habitat specialists), being abundant in cold, clear streams (<12°C) with abundant cover, especially large amounts of coarse woody debris. The distribution of bull trout is a good index of available habitat for other salmonids within the basin. Other indicators can be derived through inspections of community interaction webs.

Ecological Function. Function within a community can be identified using a variety of criteria, including (1) morphological characteristics, (2) trophic relationships within the food web, (3) distribution and habitat use (stenotopy vs. eurotopy), (4) relative abundance (e.g., dominance, scarcity), and (5) effect upon habitat (e.g., removing benthic algae, converting CPOM to FPOM, rearranging substrates by spawning, affecting the distribution of detritus, etc.). You can assign functional groups as variables or identify the variables by species. Determine whether or not you can use qualitative information from the literature and regional texts on fish faunas. Some examples with excellent natural history information are Tomelleri and Eberle (1990), Moyle (2002) and Wydowski and Whitney (2003). If you do not have sufficient information, then you have uncovered a data gap that needs research! In the meantime, you can use reasonable, more general, placeholders. For example, simple observations can reveal that bluegills (*Lepomis macrochirus*) feed on small invertebrates.

III. SPECIFIC METHODS

General Recommendations

Consult Section 1 of this book (Chapters 1–6) for methods to conduct the physical habitat inventory. Only details and protocols on the biological aspects will be presented here. Strategies for documenting field data are for project success. Some words of advice:

1. Make sure that header files of data sheets for physical and biological sampling are coordinated so that they both link back to the same sites and dates.

2. Rite-in-the-Rain® paper doesn't dissolve when wet, break when dropped, short out when dropped in the water, or run out of batteries. Written (actually all entries should be printed) entries on data sheets may take longer than digital methods to enter data, but provide a trace to the original data set. It is too easy to make a "fat-finger" mistake on a field computer or PDA or to accidentally dump a huge file (e.g., 500 way-points on a GPS unit).
3. Use #2 lead pencils in the field.
4. Two persons should check for entry errors in the electronic database.

A. Basic Method 1: Fish Population Estimation

Visual Estimation by Snorkeling. This method works well in streams with good water clarity and sufficient depth to allow snorkeler mobility. However, it is limited to fishes that do not dwell in interstitial spaces within the substrate. Population estimates are based upon accurate counting (Helfman 1983). Visual estimates can be done quickly and considerable stream length can be surveyed in a short amount of time, but errors occur when fish are double-counted or missed. Visual counts and the depletion method can provide similar results, but you must account for factors that affect detection (Hillman *et al.* 1992, Roni and Fayram 2000, Thompson 2003). Take care to account for inter-observer variation (see Hankin and Reeves 1988), including the diver's range of vision. A realistic calibration was devised by C. Zimmerman (USFWS, Juneau, AK), who realized that motion is the first cue of fish presence. He removed the treble hooks from a fishing lure that was suspended in the water from a stake with monofilament line. Fish lures simulate the motion of a swimming fish — the lotic equivalent of using a Secchi disk to measures the degree of water transparency in a lake. The lure is placed upstream from the diver who then proceeds to move upstream to find it. When the diver sees the lure, a marker is placed on the substrate at that location (such as a fishing bobber anchored with a fishing sinker). A measuring tape is then used to measure the distance from the point of detection to the lure. This measures the range of vision of the diver and indexes detectability of fish (but note that it works both ways, as the fish can also see you and hide, if startled). Size bias in fish detection can also be calibrated, as lures come in various sizes.

1. Obtain appropriate gear including mask and snorkel, dive gloves and booties, and wetsuit or drysuit with hood (hypothermia can set in quickly for the unprepared snorkeler).
2. Select a stream reach in which to conduct the visual estimation. Check with the public health service that the water is safe to swim. High fecal coliform counts indicate unhealthy conditions, and may indicate the presence of pathogens such as *Staphylococcus aureum*, *Hepatitis*, and *Vibrio cholera*. Obtain the proper inoculations when in doubt, and after diving clean your ears thoroughly, and gargle with an antiseptic mouthwash.
3. Beginning at the downstream end of the reach, snorkelers should move upstream simultaneously in parallel lanes, with lane widths determined by visibility and stream size. Good coordination among snorkelers is essential.
4. Fish counted (species and number) by each snorkeler can be recorded by an observer on the stream bank (trailing the snorkelers to avoid disturbing fish) or by the snorkeler using an underwater slate. A slate can be made cheaply of opaque plexiglass (~15 cm × 15 cm piece) and surgical tubing (~50 cm length). Drill a hole in a corner of the plexiglass and thread surgical tubing through the hole; tie a knot leaving equal lengths of tubing at both ends. With one end of the tubing, tie a loop

for placing around the wrist of your nonwriting hand. Insert a pencil into the other free end for recording data, and you have created an underwater slate that can be repeatedly erased with a good art eraser.

5. Data Analysis: In the snorkeling method, the raw counts form the estimate. It is important to account for errors among observers. For a subset of sites, each observer should make several repeat surveys of the same site. Variation in counts by each observer and among all observers should be considered when accounting for sources of error. Errors due to visibility can be detected by setting out a known number of fish mimics (Zimmerman's modified fishing lures above) in places of different water clarity or amounts of cover.

Peterson Mark-and-Recapture Estimates. Either electrofishing or seining techniques can be used to capture fish for use in mark-and-recapture estimates.

1. Block the upstream and downstream ends of the stream reach to be sampled with block seines (0.5 cm stretch mesh). Be sure to weight the bottom of the net with rocks to prevent fish escape, and keep the top of the net above the water level.
2. Make one pass through the habitat to collect fishes. Place them in an aerated bucket or a live car (a perforated holding container to be placed in the stream).[1]
3. Anesthetize fishes with either Clove Oil[2] or Tricaine Methanesulfonate (MS-222),[3] realizing that MS-222 is a hazardous substance.

Solution Use	Clove Oil	MS-222
Stock solution	10 mL Clove Oil/ liter H_2O	20 g MS-222+ 50 g $NaNCO_3$/liter H_2O
Anesthetizing solution	2.5 mL stock solution/ liter H_2O	2.5 mL/liter H_2O
Euthanizing solution	10 mL stock solution/ liter H_2O	10 mL/liter H_2O

4. Identify the fish to species. Measure each fish for fork length (nearest mm) and weight (nearest g) using a measuring board and field balance.
5. Mark the fish with a fin-clip using surgical scissors. In general, the tips of pelvic, pectoral, or the upper and lower tips of the caudal fin are clipped. The idea is to avoid impairing locomotion to any great degree. Small amounts of tissue can grow back within weeks and the marks are easily detected. For fishes with adipose fins (i.e., salmonids, trout-perch, and ictalurids), no impairment will result from clipping the entire fin.
6. Gently release fishes and wait overnight for fish to recover.

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[1] Make sure to keep the water cool and aerated. Change the water frequently if a bucket is used. Alternatively use a mesh laundry basket with a tight weave as a live car. Weigh it down with cobble and cover it with a net or tarp to prevent fish from escaping.
[2] Clove oil is low in toxicity and not carcinogenic as far as is known.
[3] Both formalin and MS-222 are toxic and carcinogenic. The utmost care should be followed in their use. Gloves and protective eyewear should be used when handling these substances and exposed skin should be protected using a silicon lotion. Do not dispose of formalin in the field.

7. Conduct a second sampling in the same reach to recapture fish, allowing sufficient time to elapse for fishes to disperse.
8. Data analysis: Mark and recapture estimates are calculated after fish are recaptured and counted after the second sampling effort. The population estimate N and its variance V(N) (from the binomial distribution) are, respectively:

$$N = (M \times n)/m \qquad\qquad (22.2)$$

and

$$V(N) = n/(M \times N) \times (1 - (M/N)) \qquad\qquad (22.3)$$

where M = number of marked fish, n = number of marked plus unmarked fish caught in the second sample, and m = the number of marked fish recaptured.

Removal/Depletion Estimate.   The removal or depletion estimate assumes that in a closed population, successive sampling efforts will result in lower catches for the same amount of effort (catch-per-unit-effort, or CPUE) because there will be fewer fish to catch. Think of a person wearing a blindfold dip-netting fish from an aquarium. As each successive pass reduces the concentration of fish, the number of fish caught on successive passes of the net will decline. All fish do not need to be caught if the depleting trend is strong because you can extrapolate to predict the total number of fish you would have caught when the CPUE became 0 (i.e., the point at which you have completely depleted the population).

1. As in the mark-recapture technique, the assumptions require that the upper and lower borders of the sampling reach of stream be blocked (closed population) to prevent movement of fishes in out of the study area.
2. Using a seine or electroshocking gear, make three separate and sequential passes within the blocked stream reach. Keep the effort (e.g., number of people electroshocking, time spent, etc.) constant among passes.
3. Following each pass, put the captured fish into a separate bucket or live car marked with the pass number. Consider each pass a catch at time i.
4. Identify, count, measure, and weigh fishes, retaining the information for each pass. The catch for passes 1, 2, and 3 will be denoted as n_1, n_2, and n_3, respectively (Table 22.1).
5. The cumulative catch for passes 1, 2, and 3 will be, n_1, $(n_1 + n_2)$, $(n_1 + n_2 + n_3)$, respectively. We will denote them as C_i, where i = 1, 2, 3.
6. Data analysis: Removal or depletion estimates can be made by visual extrapolation of CPUE (*y*-axis) vs. accumulated catches (*x*-axis; Figure 22.1). The population estimate N also can be calculated using simple linear regression and determining the *x*-intercept for the cumulative catch:

$$CPUE = intercept_y - slope \times \Sigma C_i \qquad\qquad (22.4)$$
$$\text{and when } CPUE = 0, N = intercept_y/slope$$

Other approaches to making depletion estimates are discussed by Riley and Fausch (1992).

TABLE 22.1	Data Worksheet for Making Depletion Population Estimates.

Page_____of_____
Date Recorder:_____
Team Members:_____
Date:_____ Basin:_____ Subbasin:_____
  Valley Type:_____
Stream:_____ Order:_____ Stream Gradient:_____
  Reach type:_____
Site Code:_____ GPS (UTM):_____northing
  _____easting
County:_____ Township:_____
  Site Location:_____
Channel Unit (e.g., pool, riffle, other):_____
Unit Length:_____(m) Unit Average Width:_____(m) Maximum depth:_____(m)

Species:			
Axis	Pass 1 (# fish)	Pass 2 (# fish)	Pass 3 (# fish)
y (catch)			
x (sum of catches)			

Species:			
Axis	Pass 1 (# fish)	Pass 2 (# fish)	Pass 3 (# fish)
y (catch)			
x (sum of catches)			

Species:			
Axis	Pass 1 (# fish)	Pass 2 (# fish)	Pass 3 (# fish)
y (catch)			
x (sum of catches)			

Species:			
Axis	Pass 1 (# fish)	Pass 2 (# fish)	Pass 3 (# fish)
y (catch)			
x (sum of catches)			

Species:			
Axis	Pass 1 (# fish)	Pass 2 (# fish)	Pass 3 (# fish)
y (catch)			
x (sum of catches)			

FIGURE 22.1 Depletion estimation of fish population size. Extrapolation to the *x*-intercept will be the estimate of the population. Note that the scales on this graph do not allow visual extrapolations on this example. The inset is the estimate obtained by linear regression.

B. Basic Method 2: Evaluating Structure in Fish Assemblages

Measuring Taxa Richness and Diversity. First, you should evaluate community structure. Basic descriptors of biological assemblages are abundances, taxa richness, and combination measures, generally categorized as diversity indices. A few of these measures are used in this exercise; they were selected based on their ability to discriminate among groups and ease of interpretation. Abundance is straightforward, either as a summation of all organisms or as numbers represented by particular groups. Taxa richness can be expressed as the number of taxa at various levels (species, genera, families). Diversity is a measure that combines richness with relative proportions contributed by each taxon.

1. A simple and informative way to examine these relationships is to create histograms of abundance by species. Species are listed on the *x*-axis and abundance is displayed on the *y*-axis. Abundance can be expressed in units of mass (g) or as population counts (N).
2. Traditionally, ecologists have used diversity indices based on information theory. The index H′ is a measure of uncertainty. The basic concept was derived by Shannon (Shannon and Weaver 1949) and has been modified several times since. It was proposed that the community would become more stable as species increased and their relative abundances became more equitable (i.e., even) because alternative trophic pathways would buffer the system. Unfortunately, many use this measure to examine specific taxocenes, thereby making this assumption irrelevant. After the initial flush of studies during 1960s–1970s, the meaning of H′ has been called into question (Ludwig and Reynolds 1988, Kwak and Peterson in press). Despite these misgivings, this index still has many adherents as it is the most widely used

diversity index in aquatic biology (Kwak and Peterson in press). We suggest that H′ is best used as a simple summary statistic to compare similarity among sites. The Hill's Diversity modification of H′ is among the easiest to interpret ecologically and is calculated as:

$$H' = -\Sigma[(n_i/n)\ln(n_i/n)] \tag{22.5}$$

where n_i is the number of individuals in taxon i; and n is the total number of individuals in the sample.

3. Calculate H′ for two sites of interest. Compare the graphical technique above and H′ for ecological insight. After you identify the function of each species, rerun the analysis.

Comparing Communities. In many instances, an investigator may wish to compare communities (e.g., Matthews 1986, Matthews *et al.* 1988). As an illustration, Matthews (1986) evaluated the effect of a catastrophic flood on an Ozark stream community by comparing community structure through time (i.e., before the flood and two periods after the flood). A wide variety of similarity indices can be used to compare assemblages. Two indices used in many studies are suggested for this exercise.

1. Compare two communities using Jaccard's index. Jaccard's index (C_J) is simple but does not account for abundances of species (Southwood 1978):

$$C_J = j/(a+b-j) \tag{22.6}$$

where j is the number of taxa found in both sites; a, the number of taxa in Site A; and b, the number of taxa in Site B.

2. Compare two communities using the Morisita-Horn index (C_{MH}), which takes into account both taxa richness and abundance. However, C_{MH} is highly sensitive to the abundance of the most abundant taxa (Wolda 1981):

$$C_{MH} = [2 \times \Sigma(an_i bn_i)]/[(da+db)aN \times bN] \tag{22.7}$$

where aN is the number of individuals in Site A; bN, the number of individuals in Site B; an_i, the number of individuals of the ith species in Site A; bn_i, the number of individuals of the ith species in Site B; $da = \Sigma an_i^2/aN^2$, and $db = \Sigma bn_i^2/bN^2$.

C. Basic Method 3: Identification of Functional Groups Using Morphological Inferences

Once the parts of the ecosystem have been identified, the next step is to figure out how they function. Anatomical analysis can help. Microhabitats and feeding associations can be difficult to determine when waters are murky because observations are difficult. Moreover, place of capture may not be revealing as fish may have been trying to evade the fishing gear. Anatomical structures can provide strong hints (see also Chapter 26). The presence of gustatory barbels, for instance, is associated with fishes inhabiting murky waters. The position of the mouth can indicate where the fish gathers its food. Top feeding fishes have a mouth near the top of its skull (superior). In fishes such as killifish this is accentuated because the mouth is also slanted upwards (superior-oblique). Midwater feeders or feeding generalists often have mouths at the anterior, terminal position. Rainbow trout and bluegills have terminal mouths. Benthic fishes such as longnose dace and suckers have mouths in subterminal and inferior positions, respectively. The size of the prey may be estimated by measuring the area of the mouth using two dimensions: mouth width (the distance between the left and right corners of the lower jaw) and mouth gape (the greatest distance between the upper and lower jaws when maximally distended). Calipers will greatly facilitate measurements. Use the formula of the ellipse to determine mouth area:

$$\text{Mouth Area} = (\text{major axis} \times 0.5) \times (\text{minor axis} \times 0.5) \qquad (22.8)$$

Diet also can be inferred from body parts. Herbivore-detritivores can have specialized mouth parts that scrape and teeth may be molariform or spatulate in shape (check pharyngeal teeth, too!) to break down plant cell walls. The gastrointestinal system may have grinding structures in the pharynx and an extremely long intestine to increase absorptive efficiency of food that is high in fiber, but low in calories, proteins, and fats. Invertivore-carnivores have teeth designed to seize and penetrate prey and intestinal tracts that are shorter than those of herbivore. Some predators have specialized digestive structures such as pyloric caeca, which secrete proteolytic enzymes.

The shape of the body, curvature of the tail, and streamlining are clues to habitat use. Benthic fishes often are dorsal-ventrally compressed for close adherence to the substrate. Fishes that are laterally compressed, but broad, such as white crappie or bluegills are found in slow-water habitats. Fishes dwelling in fast water are more streamlined. Streamlining can be determined using the Fineness Ratio.

$$\text{Fineness Ratio} = \text{standard length/maximum body depth} \qquad (22.9)$$

Standard length is measured from the tip of the snout to the end of the vertebral column, approximately at the base of the caudal fin. A Fineness Ratio of 4.5 is optimal for hydrodynamic efficiency (see Scarnecchia 1988). Fishes above (eel-like fishes) and below (bluegill-like fishes) that ratio are less hydrodynamically efficient. See Chapter 26 in this book for further discussion of morphological attributes of fish.

D. Basic Method 4: Identifying Functional Groups from Feeding Habits and Habitat Use

Patterns. Grouping fishes into functional groups or guilds helps you to conceptualize how niche space is utilized. There is seemingly no end to characteristics that can be used and there is no single standard protocol. However, here are some helpful hints.

1. Organize the classification into a matrix. The elements of the matrix are (a) habitats used and (b) feeding habits. The information for these categories will be obtained from, in order of priority, fieldwork, scientific journals, and regional faunal guides.
2. Using a spreadsheet, organize the habitat aspect of the functional group, nested within the spatial hierarchy. The example in Table 22.2 illustrates functional groups for a midchannel pool. From this table, the following groups can be identified based on their food habits and use of space: (1) surface-midwater invertivore; (2) midwater-substrate invertivore; (3) midwater-substrate invertivore-piscivore; (4) benthic invertivore; (5) benthic detritivore-herbivore; (6) benthic-hyporheic invertivores; (7) benthic-hyporheic invertivore-piscivore; and (8) meiofaunal predators in lateral edge habitats.
3. Use this approach to compare ecological organization among habitats at different spatial scales. Note that several species can be members of a functional group and that a species can be a member of different functional groups as their niche changes through ontogeny. Greater detail and subdivisions can be added to the functional groups, but we suggest keeping the categories broad. As the habitats are georeferenced, details such as discharge, temperature, dissolved oxygen, and elevation will help in describing species associations.

E. Advanced Method 1: Signed Digraphs and Linking Structure and Function

Loop Analysis. Thus far, we have concentrated on understanding how individual species may function. To understand how the community functions, we need to make explicit links from one species to the next. The signed digraph is a formal, graphical mathematical approach that can help make such linkages. This qualitative approach has appeal for several reasons. It is especially handy when there are gaps in quantitative data by which interaction strengths are specified. The strength of the qualitative approach derives from good descriptive studies that are useful in model building. In short, if you have a solid understanding of natural history and you can draw a model, you can use this approach.

Levins (1973) adapted this approach to examine communities, calling it "Loop Analysis" because interactions among community elements form feedback loops. Feedback can be described qualitatively as positive (+), negative (−), or none (0). All ecological interactions among species and community components can be described using these expressions (Figure 22.2). For example, predator-prey and parasite-host relations can be described by connecting a negative link from the predator (or parasite) to the prey (or host). The loop is completed by connecting a positive link from the prey (or host) to the predator (or parasite). In terms of population dynamics, prey benefit population growth of the predator (a + link), but predators decreases prey populations (a − link). Signed digraphs were so named because the interaction links are signed (+, −, 0) and the feedback has direction, hence di(rected)graphs.

TABLE 22.2	Example Worksheet for Identifying Functional Groups from Feeding and Habitat Use Patterns of Fish Observed or Collected from the Field.				
Basin	**SubBasin**	**Stream Order**	**Valley Segment**	**Stream Segment**	**Habitat Unit**
Cottonwood Creek	Rock Creek	4	alluvial	2% gradient	mid-channel pool
	Microhabitat Unit	**Species**	**Life Stage**	**Food Habits**	
	Surface-midwater	chinook salmon	Juvenile	Invertivore	
		redband trout	Adult	Invertivore	
			Juvenile	Invertivore	
	Midwater-substrate	redside shiner	Adult	Invertivore	
			Juvenile	Invertivore	
		pikeminnow	Adult	Invertivore/fish	
			Juvenile	Invertivore	
	Benthos	speckled dace	Adult	Invertivore	
			Juvenile	Invertivore	
		bridgelip sucker	Adult	Detritus/Diatoms	
			Juvenile	Invertivore	
		mountain whitefish	Adult	Invertivore	
	Benthos-hyporheos	longnose dace	Adult	Invertivore	
			Juvenile	Invertivore	
		torrent sculpin	Adult	Invertivore/fish	
			Juvenile	Invertivore	
		Paiute sculpin	Adult	Invertivore	
			Juvenile	Invertivore	
	Lateral-edge habitats	redband trout	YOY	Meiofauna	
		redside shiner	YOY	Meiofauna	
		speckled dace	YOY	Meiofauna	
		suckers	YOY	Meiofauna	
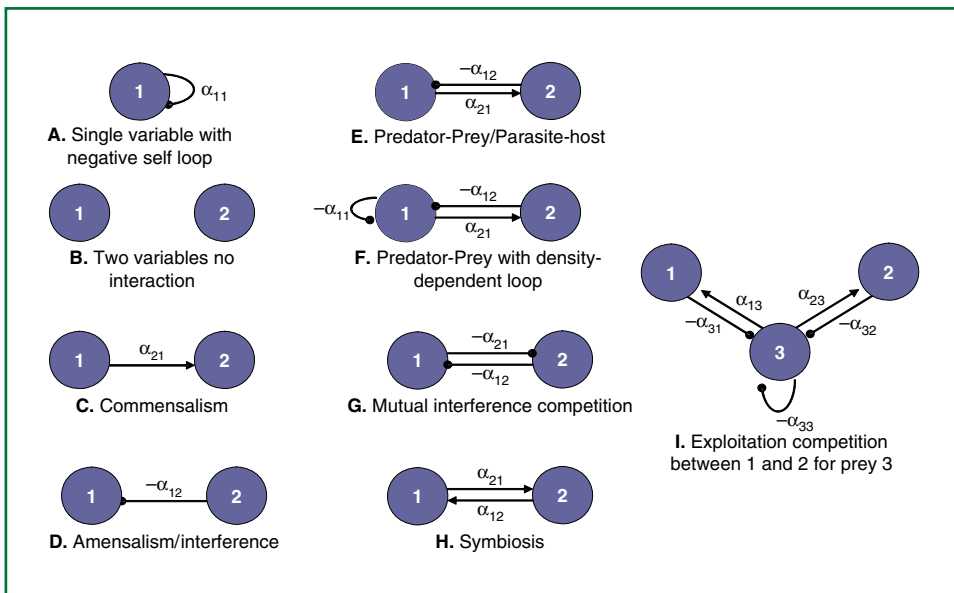
FIGURE 22.2 Ecological interactions as expressed by signed digraphs. Arrows point to the effects from one species to the next. Pointed arrows (+), Blunted arrows (−). α_{ij} denotes the effect of species j on species i.

A loop is defined as a path leading to and from a node (or variable) and its return to the starting point. By convention, no more than one link can go from one variable to the next. This convention appears to create a problem when two species interact in several ways (this is actually rather common). For example, a species may be a predator of another species at one life stage, but compete with it at another. This conundrum can be handled in two ways: (1) express the relationship as the net feedback between both species (i.e., lump both interactions into a single loop; see Figure 22.2E) or (2) partition the relationship explicitly into two relationships by life stages (Figure 22.3B). The second option treats each life stage as a separate species (cf. ecological species). A circular feedback loop is called a self-loop. When it is negative, it denotes that the species (variable) is limited by resources not explicitly described in the model. These resources may be nutrients, other prey, sunlight, oxygen, territorial limitations, agonistic interactions of a behaviorally regulated population, or other factors. A positive self-loop acts as an amplifier, reinforcing the signal. An example is panic; it tends to build and increases the level of anxiety. Naturally, this leads to instability.

What can you learn from signed digraphs? Through visual inspection, you can examine niche partitioning. As an illustration, consider a stable system comprised of fish, herbivores, and algae (Figure 22.3B). An asymmetrical feeding overlap exists between H1 and H2. H1 diet overlaps with H2 by 100%, but H2 overlaps with H1 by only 50%. It is difficult to determine the degree of overlap between H3 and the others because the negative self-loop indicates that it is limited by resources not explicitly described in the model. If exploitation competition for resources is intense, how do they coexist? Why isn't H2 squeezed out by H1 and H3? The keys are predation by fish on H1 that prevents it from displacing H2, and H3 has other resources supporting it. In a sense, niche description of H1 also includes its predator, not just its dietary habits.
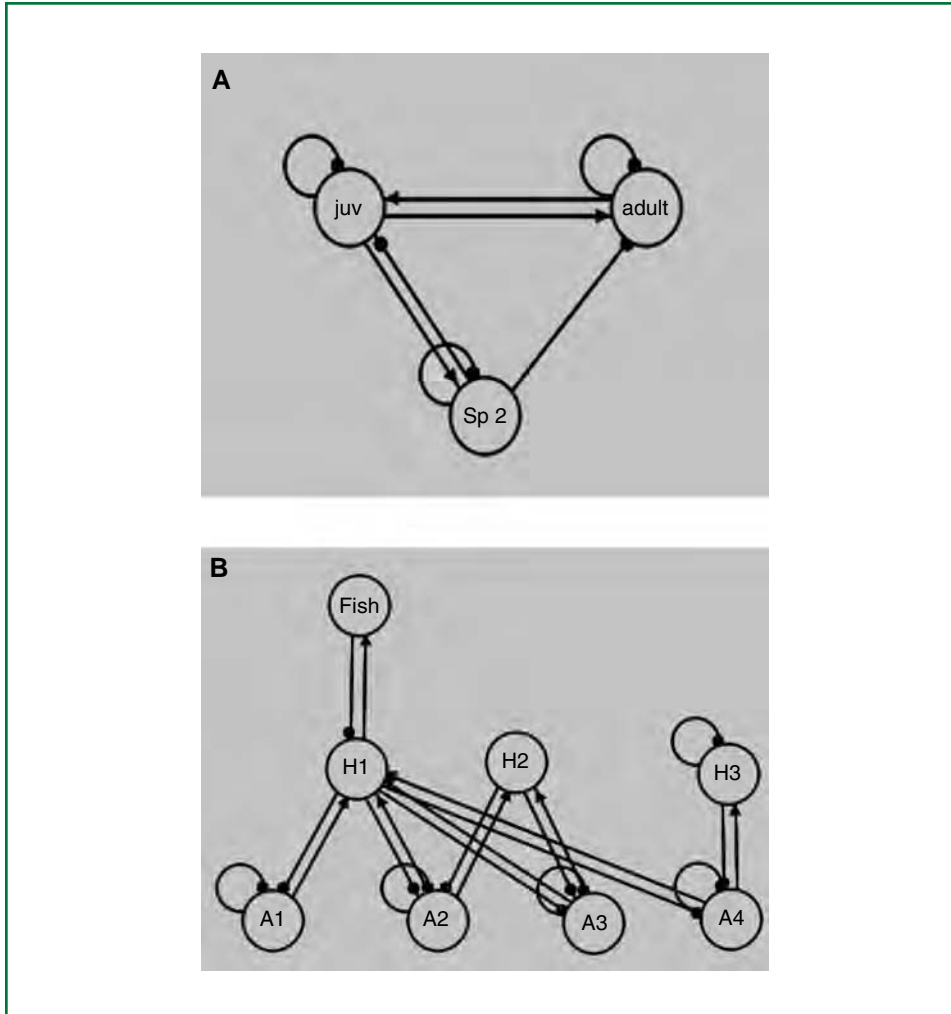
FIGURE 22.3 Theoretical digraphs. A. Species 1 is partitioned into 2 life stages. Note that the interactions between the two stages is positive and that each has a negative self-effect, signifying that each is limited by resources not explicitly described in the graph. B. H1 has trophic overlaps with H2 and H3. H2 and H3 have negative indirect effect effects on fish through the food web. See text for details. A = algae, H = herbivore.

Indirect effects along the food web also can be traced from the digraphs. In contrast to *direct effects*, which have interaction links connecting two variables, indirect effects involve variables that are not directly linked to each other but are connected on the same pathway of interactions with other variables. For example, fish are affected by H2 and H3, although there is no direct connection to them. The two herbivores affect the abundance of fish prey (H1) by grazing on common algal resources (A2 and A4). Subsequently an increase in H1 affects fish positively. Variables in the digraphs do not have to be species but can be guilds or functional groups or storage compartments such as detritus. Describing the community using signed digraphs will help researchers

to understand the community in a formal, structured format. Mathematically, much more can be gained from the technique; however, this requires knowledge of matrix algebra.

For the mathematically inclined, more can be gained from Loop Analysis if you know matrix algebra. You can determine local stability and obtain qualitative predictions of how the entire community will respond to either positive or negative input to any member of the community. How is this possible from qualitative information? The signed digraph can be converted into a matrix A. Each element of the matrix, a_{ij}, describes the effect of the species j on species i and is assigned the values of $+1$, -1 or 0, when the interaction is positive, negative, or none, respectively (Quirk and Rupert 1965). Qualitative values have now taken numerical form in a matrix of community interactions. We can now use matrix algebra to (1) determine whether the system is locally stable and (2) predict changes in the standing crops of every member of the community at some future state when one of the community members is perturbed. These qualities make Loop Analysis a very attractive tool for managing ecosystems and communities (Bodini *et al.* 1994, Li *et al.* 1999, Bodini 2000, Hulot *et al.* 2000, Castillo *et al.* 2000, Dambacher *et al.* 2002a, 2003a, b, Ramsey and Veltman 2005). Very briefly, local stability of a community is conferred by negative feedback through the system. If the system is stable then predictions are possible by asserting Cramer's Rule (see Dambacher *et al.* 2002c). If the system isn't stable, predictions concerning the response to a press to the system are moot. A press is a sustained change imposed on the system and differs from a pulse, which is an acute change. The predictions are are expressed as qualitative changes of standing crops $(+, -, 0)$ among members of the community. Among the many advantages are that predictions from Loop Analysis account for indirect ecological effects, which influence outcomes in ways that are frequently not intuitive. More mathematical details and freeware can be downloaded from the following sources: *Ecology*'s electronic database (see Dambacher *et al.* 2002b, c, d), our website (http://www.jambrosi.com), or the website of a colleague, Antonio Bodini (http://www.dsa.unipr.it/~alle/ena/). For more details concerning the application of matrix algebra for ecological analysis read Case (2000).

We can illustrate the predictive power of Loop Analysis by analyzing two classic papers. Hairston *et al.* (1960) argued that only plants were self-regulated, that herbivores were regulated by their predators, and that predators are regulated by their prey (Figure 22.4). Such a community passes the Routh-Hurwitz Criteria as stable and therefore we can test the reactions of the community to a press. Each press exhibits trophic cascading, but the pattern differs depending upon the source of input. If we create good conditions for the predator, the standing crops of herbivores decrease resulting in greater standing crops of plants as they experience less grazing pressure. Conditions favoring herbivores increase predators and although there is greater production of herbivores, there is no change in standing crop of herbivores. In other words, herbivores are eaten as fast as they can reproduce. As there is no change in the standing crop of grazers, there is no change in that of plants. If plants are stimulated to grow, they increase their standing crops. This results in an increased standing crop of predators, but no change in the standing crop of herbivores as above.

Both direct and indirect effects were observed by Flecker (1992) in experiments conducted to examine the influence of a detritivorous-algivorous fish on a tropical stream community (Figure 22.4). A inspection of this food web reveals that the detritivorous-algivorous fish (D-Fish) has a negative effect on the invertivorous fish (P-Fish), although they do not interact directly. The D-Fish competes with the food base of the P-Fish. Likewise, the P-Fish affects the D-Fish by preying on its competitor. This model is locally

Community Matrix (A)
effectors

affected	Predator	Herbivore	Plants
Predator	0	1	0
Herbivore	−1	0	1
Plants	0	−1	−1

Change in Abundance from (+) Input
Adjoint ($-A^{-1}$)
effectors

affected	Predator	Herbivore	Plants
Predator	1	1	1
Herbivore	−1	0	0
Plants	1	0	1
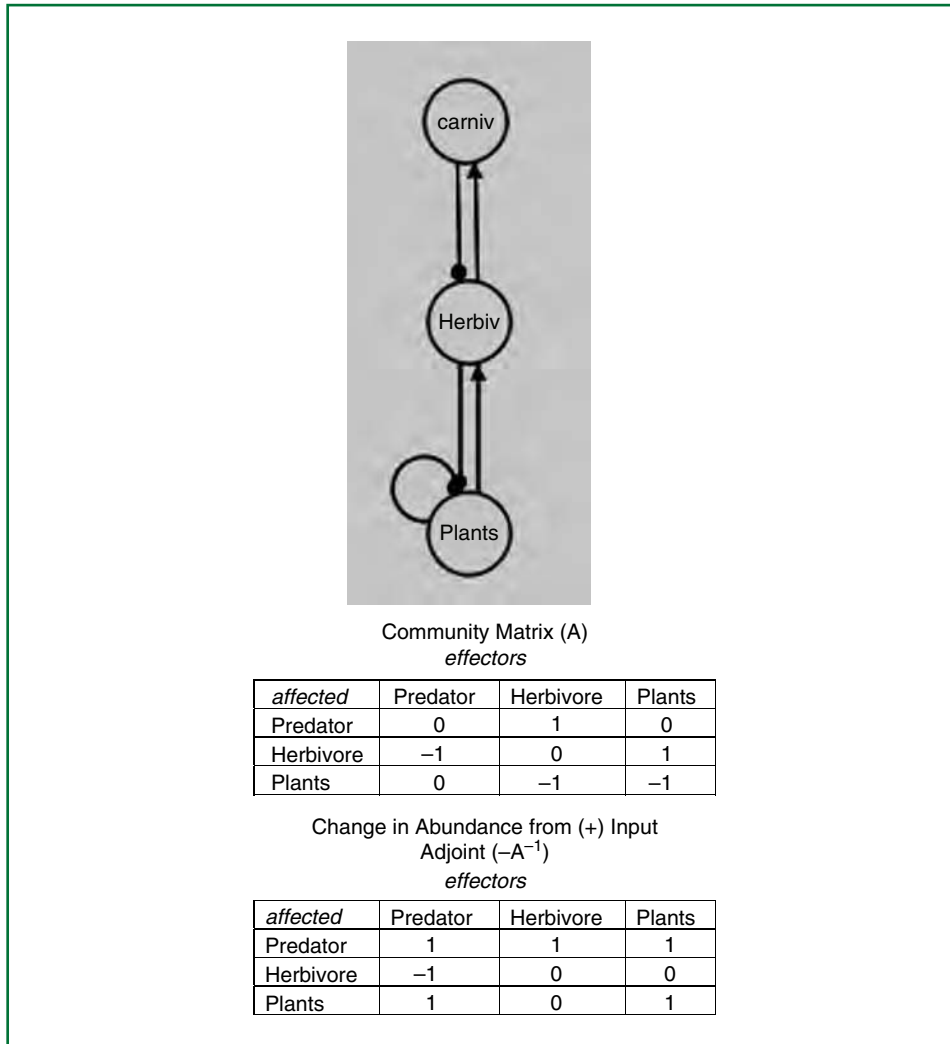
FIGURE 22.4 Signed digraph of the Hairston *et al.* (1960) model of trophic relationships. The interactions (α_{ij}) of the digraph expressed as a community matrix (effects of Component j on Component i, where i = row and j = column). The so-called table of predictions is more formally recognized as the adjoint of the negative community matrix. Changes in standing crop are predicted from a press disturbance or long term change in input. Conventionally, input comes down through the column (e.g., increases in predators cause a decrease in herbivores in column 1). Change the signs down each column to get negative (−) inputs. Note the trophic cascades.

stable and predictions of press experiments were obtained (Figure 22.5). To gain further facility with Loop Analysis, we suggest that you:

1. Read Flecker (1992) and compare the predictions made with Loop Analysis to the results of his study. Likewise, read Hairston *et al.* (1960) and determine whether or not their ideas concerning community regulation matches the output of the adjoint matrix.

Community Matrix (A)
effectors

affected	Predatory Fish	Invertebrates	Detritus-Algae Eating Fish	Detritus-Algae
Predatory Fish	0	1	0	0
Invertebrates	−1	0	0	1
Detritus-Algae Eating Fish	0	0	0	1
Detritus-Algae	0	−1	−1	−1

Change in Abundance from (+) Input
Adjoint (−A⁻¹)
effectors

affected	Predatory Fish	Invertebrates	Detritus-Algae Eating Fish	Detritus-Algae
Predatory Fish	0	1	−1	0
Invertebrates	−1	0	0	0
Detritus-Algae Eating Fish	1	0	1	1
Detritus-Algae	0	0	−1	0

FIGURE 22.5 Flecker's (1992) work on strong and weak interactions by fish guilds as they affect stream community structure in tropical streams as depicted by sign digraphs, the community matrix, and predictions of outcomes when (+) press disturbances are induced upon the system. See the text for further details. Reverse the signs for negative (−) inputs.

2. Make a signed digraph of the community at your two sites. Take advantage of work completed for other chapters (e.g., Chapters 23–26) and the scientific literature, which are sources of information useful for your model (e.g., amensal relationships between freshwater mussels and fishes, commensal relationships between beavers and fishes inhabiting beaver dams, interference competition among fishes, etc.).

3. Inspect the signed digraphs to determine functional groups, exploitation competition among species, and where indirect trophic effects may occur. Find species that are highly connected to other species. Ask what would happen if these species were to disappear. Can you spot potential keystone predators? Can you find alternative trophic pathways?
4. Compare the sites. Are the communities different? Do they operate in different ways?

Other Analyses. Multivariate techniques, such as ordinations, provide a powerful alternative for assessing assemblage structures (McCune and Grace 2002). These techniques retain information about taxa membership, establishing similarity among assemblages on several gradients. Analysis can be iterative. After significant axes and gradients are identified, taxa that respond to these gradients can be determined. Correlations between strong gradients and particular taxa establish potential indicator organisms, and can suggest further experimental work to identify key mechanisms responsible for community structure.

IV. QUESTIONS

1. How might the choice of fish enumeration method affect your interpretation of assemblage structure?
2. How might the condition of the watershed upstream or downstream affect assemblage or community structure at the study site?
3. Which of the two methods provides greater ecological insight — graphical measures of species richness and abundance, or H′? Be specific, using your data as the basis of your argument.
4. Which combination will yield the most information — using species composition or the composition of functional groups in conjunction with Jaccard's Index or Morisita's Index?
5. How does your assessment of habitat use and food habits match that of the reconstruction from the literature? How does this match up with the data from your fieldwork?
6. The negative self-loop indicates that a variable (i.e., species or functional group) is regulated by resources not explicitly described by the model. Does this make the study of assemblages easier to place in an ecological context? Why should one be cautious about its use?
7. Remove different species or variables from the signed digraph as modeling experiments. Is there a particular species that, when removed, changes community membership more than any of the others? Why?

V. MATERIALS AND SUPPLIES

Maps

   Must have: 7.5′ USGS quadrangle maps, magnetic compass
   Nice to have: GPS unit, GIS software, computer

Field Collection Permits

Official collecting permit (appropriate agency)
Institutional animal use permit (e.g., IACUC approval)

Snorkeling Gear

Wet suit or dry suit with hood
Mask and snorkel
Wading shoes
Dive gloves
Underwater slate and pencils
"Pseudo"-fish (hookless lures attached to stakes) for observer calibration

Fish Capture Gear

Buckets (3–5) for transporting and sorting fish
Live baskets (perforated holding tanks placed in the stream to hold fishes alive)
Block seines (2) (0.5 cm stretch mesh, height and length adequate for regional conditions)
Lead-lined beach seine or haul seine with bag end (0.5-cm mesh, height and length adequate for regional conditions)
or
Backpack electroshocker, safety gear (electrician gloves, rubber insulated waders), polarized sunglasses, and dip nets. Note that in some states, electrofishers must take a safety course and be certified.

Voucher Collection

10% Formalin[3]
50% isopropyl alcohol
Clove oil or buffered MS-222
Heavy museum stock labels
Pencil or India ink pen
Large wide-mouth nalgene jars
Scalpel

Note: When collection samples are made, euthanize fish in lethal solutions of either Clove Oil or MS-222. Once euthanized, preserve specimens in 10% formalin. If the fishes are to be used for dietary analysis, make an incision along the abdominal cavity to preserve stomach contents (especially important for large fishes). Sampling labels should include location, date, gear used, collectors, references to data bases, and effort. Allow specimens to fix in formalin for 72 h. Decant the formalin and soak the collection for 24 h in water. Repeat as necessary until the formalin odor is gone. Preserve the collection in 50% isopropyl alcohol. Use your institute's procedures for handling and disposing of hazardous chemicals. The fishes can then be identified, counted, and measured.

Field Measuring Equipment

50-m survey tape and wooden stakes (for determining habitat area)
Calibrated gauging staff (for measuring stream depth)
Current velocity meter (if available, for determining discharge)
Calipers
Fish measuring boards

Field balance (±1 g or 0.1 g depending on size of fishes)
Stainless steel rulers

Data Recording and Miscellaneous Supplies

Colored pencils for sketching maps
Data forms on water-resistant paper
Graph paper
Data box to store forms
Clipboard
Regional guide or key to fishes (see Appendix 22.2)

VI. REFERENCES

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VII. FOREWORD TO THE APPENDICES

The Fish Key (Appendix 22.1) in this chapter is biased toward small, wadeable streams. To construct this key, we sampled the literature from as many regions of the United States as possible, but this key still displays a heavy Mississippi Basin influence (not surprising given its large size and high diversity of fishes). To compensate for this, we scoured databases and library files to build a list of as many regional guides to freshwater fishes as possible from around the world (Appendix 22.2). As we are not linguists, some of the international listings may be quite amusing to native speakers. For this, we apologize.

APPENDIX 22.1
Key to Common Freshwater Fish Families Found in Wadeable Streams of the U. S. (modified after Eddy 1957; color drawings by J. Tomelleri)

1a. Jawless, oral cusp lined with teeth, 7 paired gill pouches, no paired fins . . . .
. . . . **Petromyzontidae (lampreys)**

1b. Mouth with upper (maxillae) and lower (dentary) jaws, 1 or 2 pairs of paired fins (pectoral and pelvic, respectively), opercular gill cover **2**

Anguillidae

Jaws
Operculum
Paired Fin

Petromyzontidae
Entosphenous tridentatus — Pacific lamprey
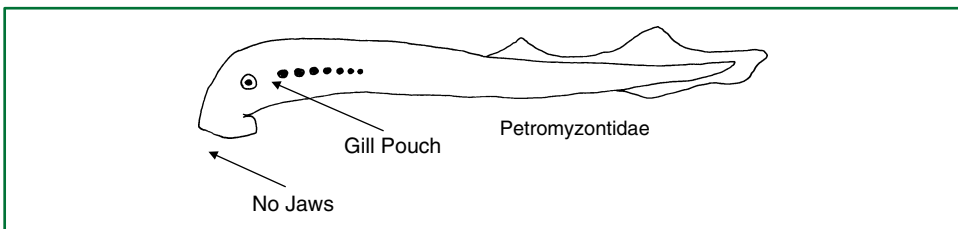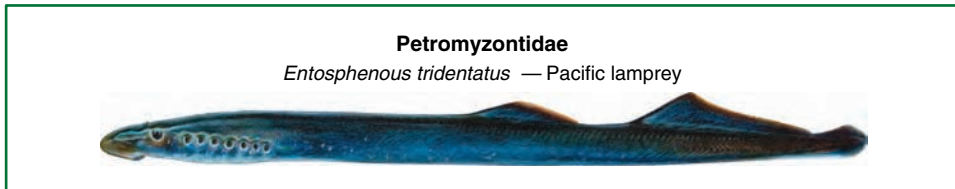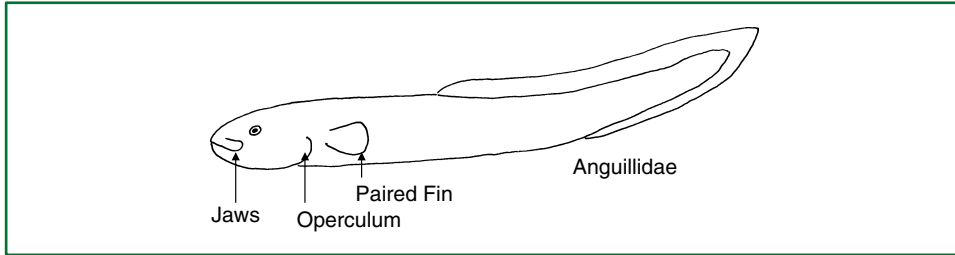
Heterocercal Tail

Homocercal Tail
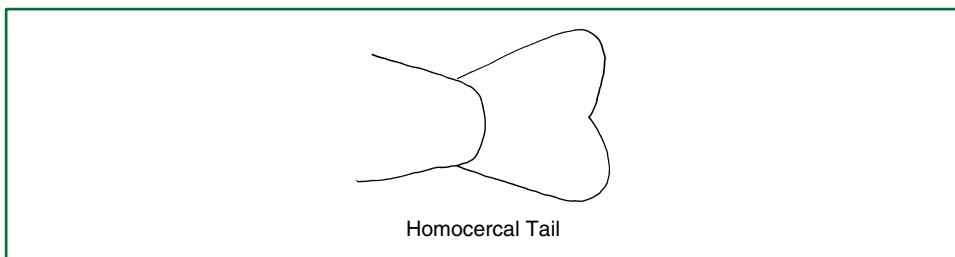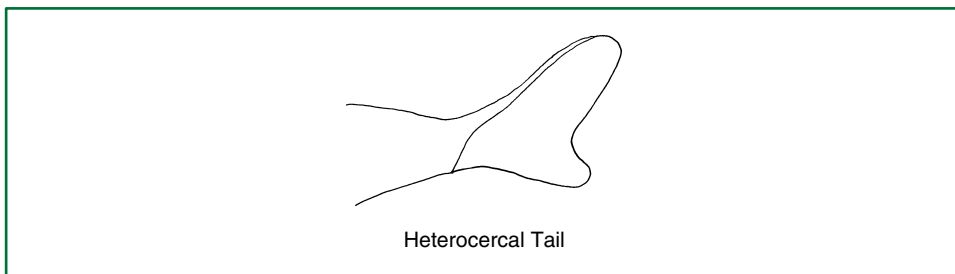
3. Body elongated (head length about 20% of body length), dorsal fin extends from approximately the lower third of the pelvic fin's extent almost to the caudal fin . . . . **Amiidae (bowfins)**

Amia calva — bowfin

4a. Pelvic fins absent, body long as in a pennant, dorsal fin long and continuous with the caudal fin . . . . **Anguillidae (eels)**

Anguilla rostrata — American eel
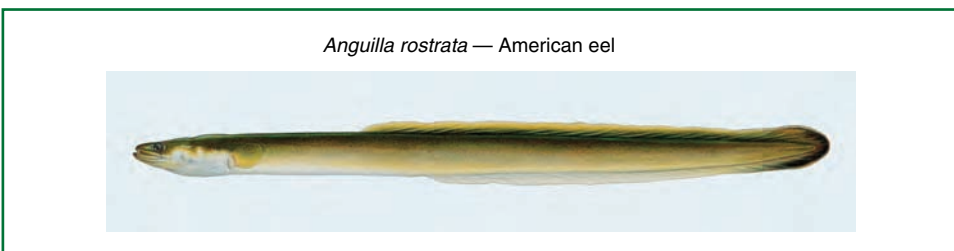
4b. Pelvic fins present, body not eel-like **5**
5a. Pelvic fins abdominal in position and near the anal fin **6**
5b. Pelvic fins jugular in position and proximal to and under the pectoral fins **16**
6a. Scales not present on head **7**
6b. Head covered with scales **13**
7a. Fins soft-rayed, without spines except for alien carp and goldfish and native desert minnows **8**
7b. Both spiny and soft rays present **12**
8a. Adipose fin and axillary processes at base of pelvic fin present **9**
8b. Adipose fin absent, axillary processes present **10**
8c. Both adipose fin and the axillary processes absent **11**
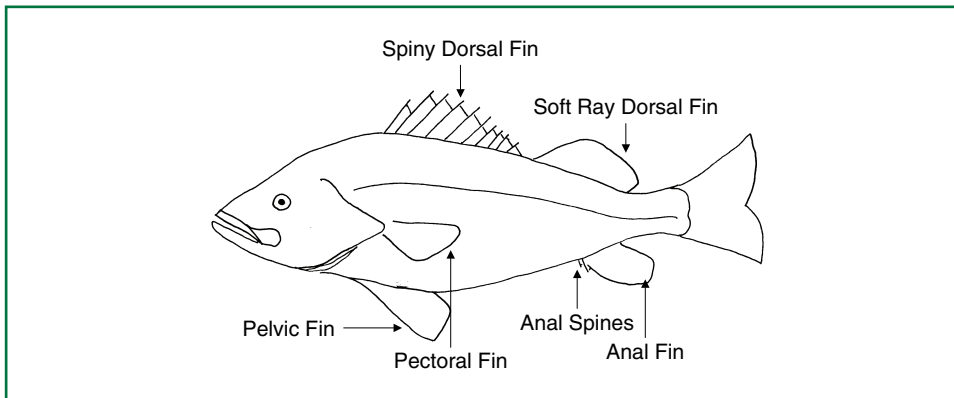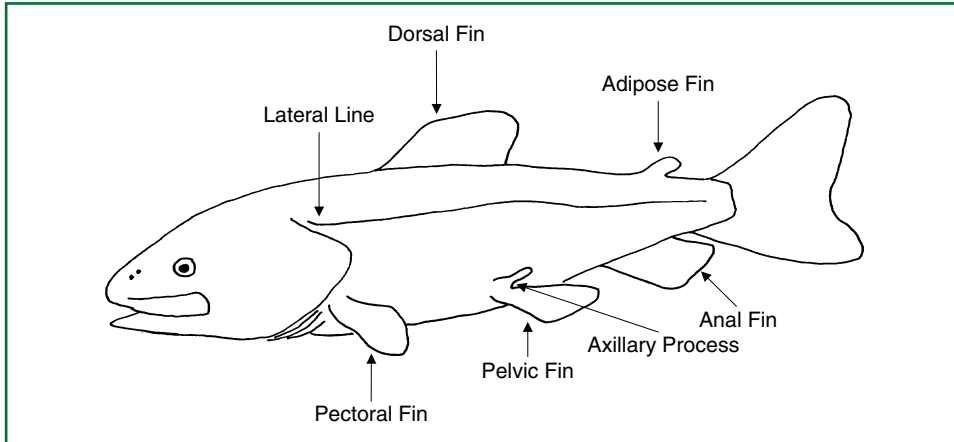
9a.   Base of dorsal fin greater or equal to head length, rays greater than 15 . . . .
. . . . **Family Salmonidae, Subfamily Thymallinae (grayling)**

Thymallus arcticus — Arctic grayling
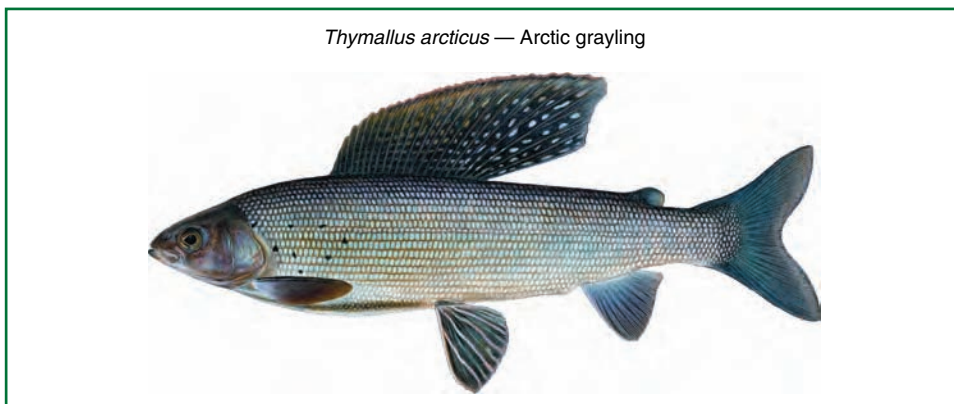
9b.   Base of dorsal fin shorter than lead length, rays less than 15 . . . .
. . . . **Salmonidae (salmon, trout, white fishes)**

Oncorhynchus tshawytscha — chinook salmon
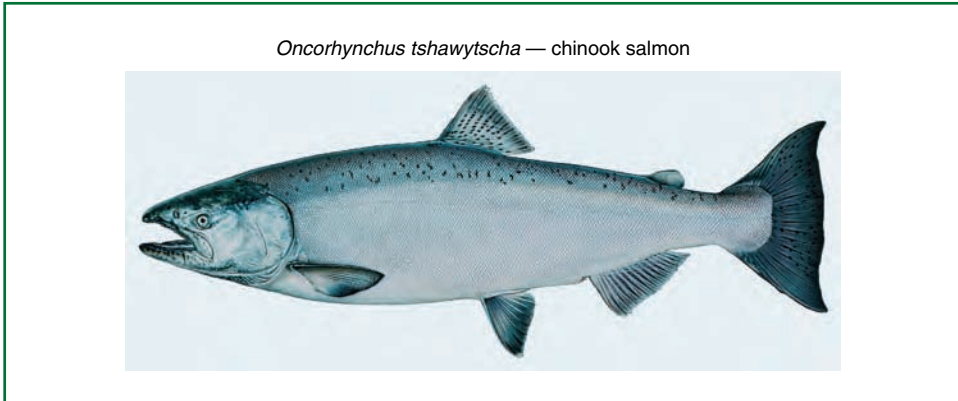
10a.   Lateral line absent, saw-tooth ventral keel for the entire abdominal length . . . .
. . . . **Clupeidae (herring, shad, alewives)**
10b.   Lateral line present, ventral keel smooth and partially present along the abdomen
. . . . **Hiodontidae (mooneye)**

Serrate Keel

Axillary Appendage

Smooth Keel

Clupeidae
Alosa aestivalis — blueback herring

Hiodontidae
Hiodon tergisus — mooneye
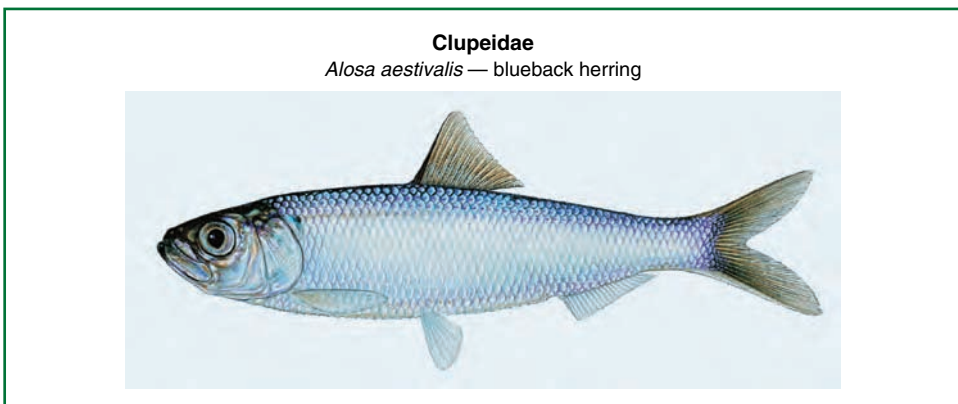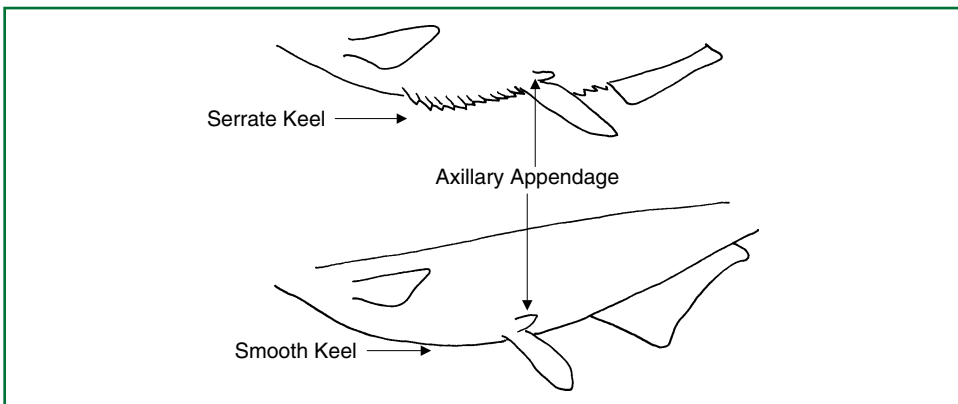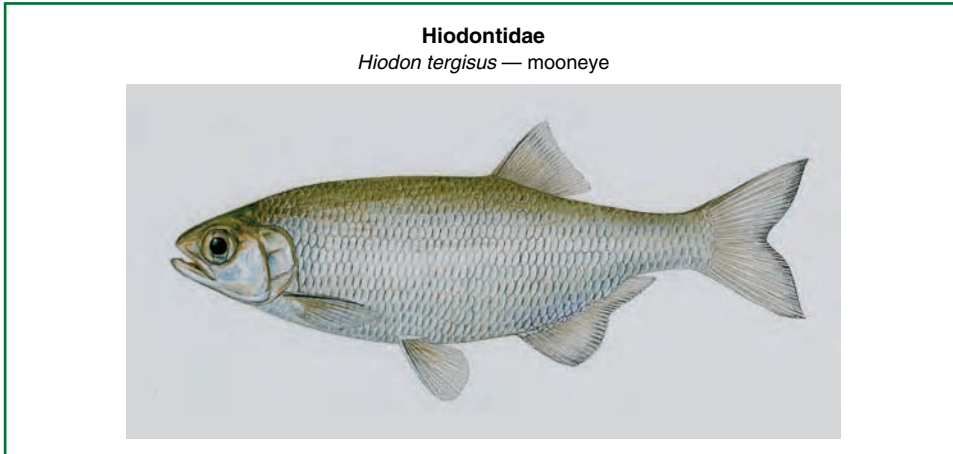
11a. Comb-like, single row of teeth on the last pharyngeal gill arch, dorsal rays usually more than 10, mouth usually thick and fleshy, especially lower lip, mouth sucker-like, usually in inferior or subterminal position . . . . **Catostomidae** (**suckers**)

11b. No more than 5 pharyngeal teeth in a single row, teeth can be in multiple rows, but no more than 10 teeth on a pharyngeal arch, dorsal rays usually less than 10 . . . . **Cyprinidae** (**minnows**)

Pharyngeal Teeth on the Last Gill Arch

Catostomidae   Cyprinidae

Catostomidae
Catostomus macrocheilus — largescale sucker
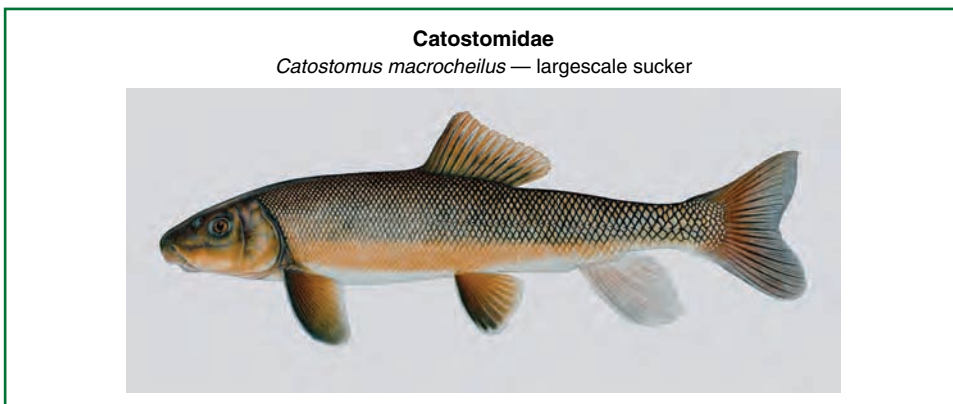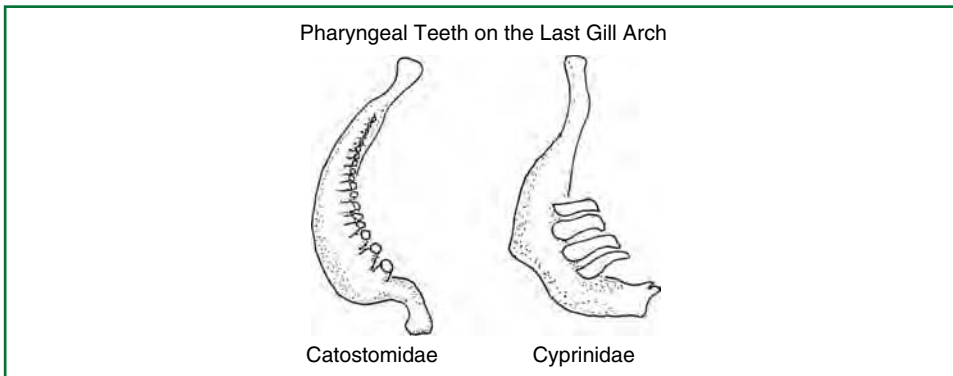
Cyprinidae
Notropis nubilis — Ozark minnow

12a. Long barbels present above and below the mouth, adipose fin present, scales on body absent . . . .**Ictaluridae** (**catfish**)

Ictalurus punctatus — channel catfish

12b. Long barbels absent, adipose fin present, scales on body present . . . .
. . . . **Percopsidae** (**trout-perch**)

Percopsis transmontana — sand roller

13a.   Small spiny dorsal fin in front of soft dorsal fin . . . . **Atherninidae (silversides)**

Labidesthes sicculus — brook silverside
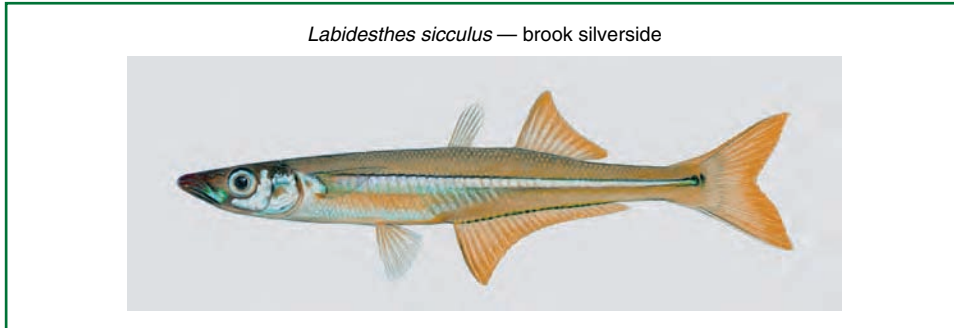
13b.   Dorsal fin single and soft rays **14**
14a.   Both jaws long, protruding like a duck bill . . . . **Esocidae (pike)**

Esox lucius — northern pike
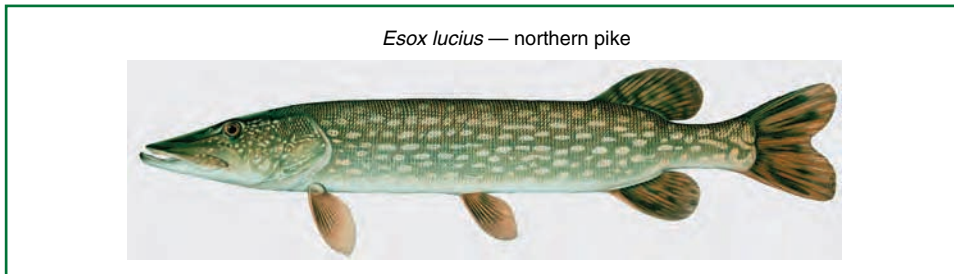
14b.   Lower jaw protruding, mouth in a superior oblique position **15**
15a.   Third ray of the anal fin unbranched, anal fin of male modified for copulation
       (gonopodium) . . . . **Poeciliidae (livebearers)**
15b.   Third ray of anal fin branched, no gonopodium in males . . . .
       . . . . **Cyprinodontidae (killifish)**

Gonopodium — Poecilliidae

Unbranched Ray   Branched Ray
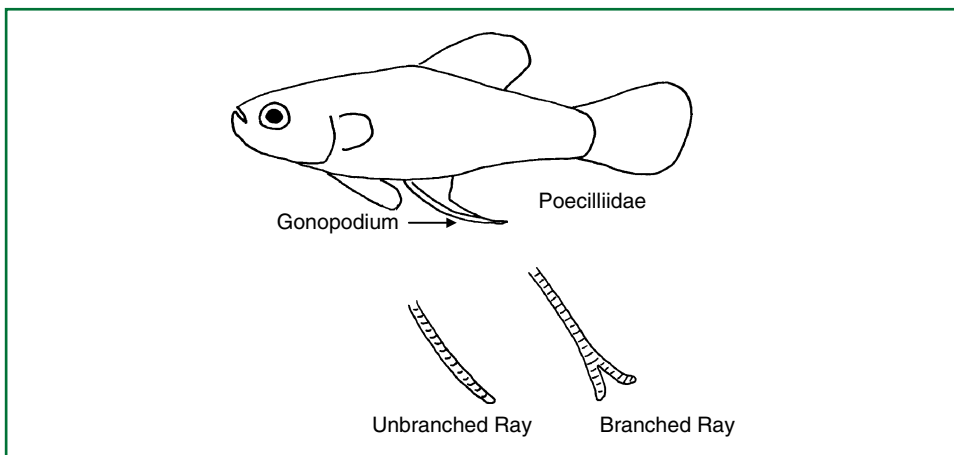
Cyprinodontidae
Fundulus zebrinus — plains killifish

Poeciliidae
Gambusia affinis — mosquitofish

Male

Female

16a.   Anus anterior to pectoral fins below the throat in adults . . . .
. . . .**Aphredoderidae (pirate perch)**

Aphredoderus sayanus — pirate perch
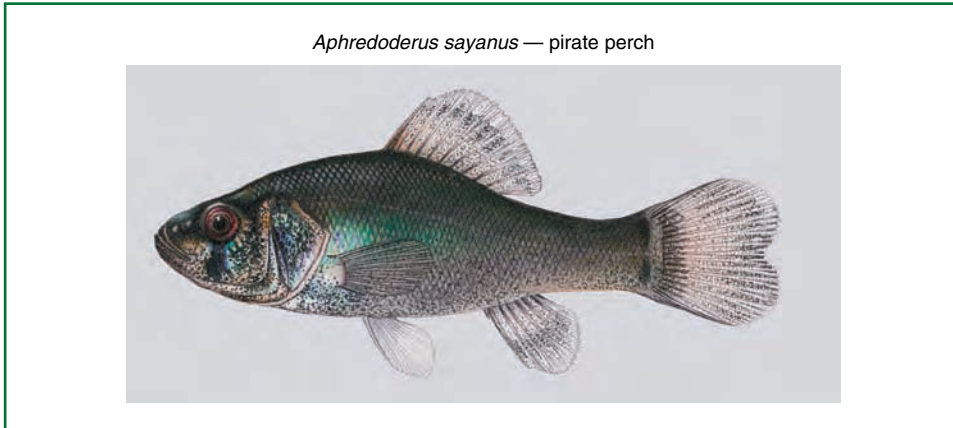
16b.   Anus posterior below pelvic fins just anterior to the anal fin **17**
17a.   Body without scales, covered with small prickly spines, or plates **18**
17b.   Body with scales **19**
18a.   Pelvic fin formed into a single spine, spines on the first dorsal fin not
connected with membrane, head laterally compressed . . . .
. . . .**Gasterosteidae (stickleback)**

Pungiteus pungitius — ninespine stickleback
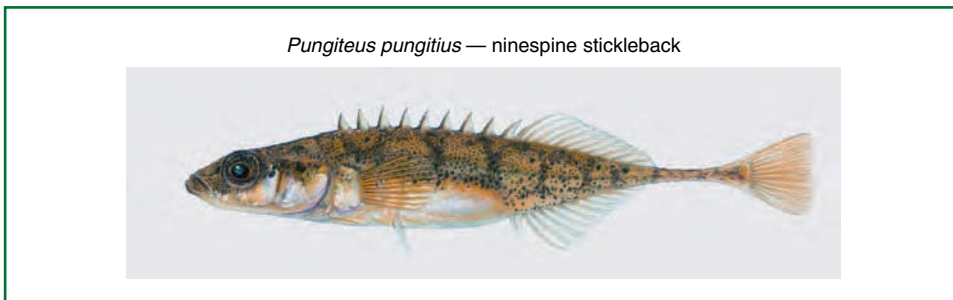
18b.   Spines on first dorsal fin connected with membrane, pelvic fins not shaped as
spines, large pectoral fins, head dorsal-ventrally compressed . . . .
. . . .**Cottidae (sculpins)**

Cottus bairdi — mottled sculpin

19a.　First dorsal fin with 6-15 spines, anal spines 3 or greater . . . .
. . . . **Centrarchidae (sunfish)**

Lepomis cyanellus — green sunfish

19b.　Anal spines less than 3 . . . . **Percidae (perch, darters, walleye)**

Etheostoma exile — Iowa darter

APPENDIX 22.2
Regional Freshwater Fish Guides by Continent and Country

Europe

British Isles

A Key to Freshwater Fishes of the British Isles with Notes on their Distribution and Ecology. Maitland, P. S. 1972. Freshwater Biological Association, Ambleside, Westside, UK.

Croatia

Prirucnik za Slatkovodono Ribarstvo. Livojevic, Z., and C. Bojcic. 1967. Savez poljoprivrednih inzenjera i tehnicara SR Hrvatske; Poslovno udruzenje privrednih organizacija slatkovodnog ribarstva "Kornatexport." Zagreb.

France

Poissons d'Eau Douce. Spillmann, C. J., and P. Lechevalier. 1961. Paris.

Germany

Freshwater Fishes. Schindler, O. 1957. Thames and Hudson Ltd., UK.

Greece

Check List of Freshwater Fishes of Greece: Recent Status of Threats and Protection. Economidis, P. S. 1991. Hellenic Society for the Protection of Nature, Athens, Greece.

Italy

Appunti sulla Fauna Ittica d'Acqua Dolce. Giussani, G. 1997. Consiglio nazionale delle ricerche, Istituto italiano di idrobiologia, Verbania Pallanza.

Portugal

Peixes dos Rios de Portugal. Almaça, C. 1996. Edições Inapa, Lisboa.

Russia (USSR)

Biologicheskie I Rybokhoziaistvennye Issledovaniia Vodoemov Verkhnei Volgi. Nikanorov, I. U. I. 1989. Gos. nauchno-issl. in-t ozernogo i rechnogo rybnogo khoziaistva (Rosrybkhoz), Leningrad.

Freshwater Fishes of the U.S.S.R. and Adjacent Countries. Ryby Presnykh Vod SSSR I Sopredel'nykh Stran. Berg, L. S. 1965. Israel program for Scientific Translations Ltd., Jerusalem. [available from the Office of Technical Services, U.S. Dept. of Commerce, Washington]

Spain

Peces Continentales Españoles: Inventario y Clasificación de Zonas Fluviales. Doadrio, I. B. E., and Y. Bernat. 1991. ICONA: CSIC, Madrid.

Sweden

Sveriges Landskapsfiskar. Nilsson, O. W., and R. Smedman. 1996. Natu och kultur. Stockholm, Sweden.

Turkey

Türkiye Tatlisu Baliklari. Geldiay, R., and S. Balik. 1999. Ege Üniversitesi Basimevi, Izmir.

Yugoslavia

Slatkovodne Ribe Jugoslavije. Vukovic, T., and B. Ivanovic. 1971. Zemaljski musej BiH-Prirodnjacko odjeljenje, Sarajevo.

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Section D

Community Interactions

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Primary Producer-Consumer Interactions

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I. INTRODUCTION

Benthic environments in streams comprise zones of high biological activity in which processes such as primary production, consumption, nutrient cycling, and decomposition predominantly occur. Primary producers and their consumers interact in this zone as they do in all ecosystems. Producers become established, grow, and reproduce, while primary consumers (hereafter termed "grazers," largely animals) ingest producer biomass to likewise grow and reproduce. Primary producers in streams consist of algae, bryophytes (mosses and liverworts), vascular plants, and some autotrophic bacteria. In most small streams, however, benthic algae are the dominant primary producers (Bott 1983, Wehr and Sheath 2003) and will grow on virtually any submerged surface, inorganic or organic, living or dead (Lamberti 1996). Benthic algae commonly found in streams include diatoms, filamentous and nonfilamentous green algae, blue-green algae (Cyanobacteria), and sometimes red algae and other algal groups (see Chapter 16). The entire attached microbial community is considered to be *periphyton* (historically called "aufwuchs" or more recently "biofilm"), of which algae are usually the main living component. The benthic surface on which periphyton grows also is sometimes used to describe the growth, such as *epilithon* (literally, "on the surface of rocks"), *epipsammon* ("on sand"), or *epixylon* ("on submerged wood").

Grazing is the consumption of living producers or their parts by primary consumers. Many aquatic animals consume periphyton, either for most of their energy intake (as with invertebrate *scrapers*; see Chapter 25) or as a variable portion of their diet (as with *omnivores*). Arguably, most aquatic invertebrates and many fish probably consume periphyton during at least some part of their lives (Lamberti 1996). The diversity of stream grazers spans a broad range of taxonomic groups, but insects, mollusks, and crustaceans are particularly important (Lamberti and Moore 1984). Among the more conspicuous benthic grazers in streams are caddisflies (Trichoptera), mayflies (Ephemeroptera), and snails (Gastropoda), and much work has been done on their grazing ecology (reviewed by Feminella and Hawkins 1995, Steinman 1996). More recently, the ecological importance of grazing by crayfish (Hart 1992, Creed 1994), shrimp (Pringle 1996, March *et al.* 2002), fish (Power and Matthews 1983, Power *et al.* 1988, Wootton and Oemke 1992, Flecker *et al.* 2002), and larval amphibians (Lamberti *et al.* 1992, Peterson and Boulton 1999) also has been recognized in a broad range of streams at many different latitudes. Regardless of the specific organisms, it is clear that many aquatic grazers consume benthic producers (Gregory 1983) and that, for some, their growth and development is linked directly to algal production (e.g., Feminella and Resh 1990, 1991, Hill 1992).

The organic matter synthesized by primary producers in streams (so-called *autochthonous* production) is a major energy source for benthic food webs. In some streams with limited riparian shading or inputs of deciduous vegetation, such as in arid lands, algal production can dominate the annual energy budget (Minshall 1978). In most streams, but particularly third-order and higher, autochthonous production constitutes a significant proportion of the energy budget (Lamberti and Steinman 1997). Middle-order (orders 3–6) streams frequently are autotrophic because light levels are high (influence of riparian shading is restricted to stream margins), water is shallow and clear (allowing light penetration to the streambed), and temperature and nutrient levels usually are suitable for benthic algal growth (Vannote *et al.* 1980, Minshall *et al.* 1985). In large rivers, internal production usually shifts from benthic algae to phytoplankton because of increased depth and turbidity, which limit light penetration to the riverbed. However, the shallow margins of large rivers can have substantial benthic primary production and abundant herbivores (Thorp and DeLong 1994). Even in small, heavily shaded streams with low algal standing crops, algae can support abundant grazer populations with their rapid turnover and high nutritional value (i.e., possessing low C:N ratios) relative to other carbon sources (e.g., detritus) and can strongly influence the structure of entire food webs (McIntire 1973, Cummins and Klug 1979, Mayer and Likens 1987).

Given the energetic value of benthic producers and their consumption by a diverse array of grazers, it is reasonable to postulate that grazers have strong impacts on plant assemblages in many streams. Indeed, many structural and functional attributes of benthic algae can be altered by grazers, but their effects are not consistent in direction or magnitude across streams, time, algal assemblages, or grazer type (Gregory 1983, Feminella and Hawkins 1995, Steinman 1996). For example, biotic factors such as grazer species, abundance, or size (Lamberti *et al.* 1987a, Steinman 1991) and algal successional state (Dudley *et al.* 1986, DeNicola *et al.* 1990, McCormick and Stevenson 1991) can each influence producer responses to consumption. However, the strength and outcome of the producer-consumer interaction also is dependent on many abiotic factors such as light (Steinman 1992, Wellnitz and Ward 2000), ambient nutrients (Rosemond 1993), substratum (Dudley and D'Antonio 1991), flow (DeNicola and McIntire 1991, Opsahl *et al.* 2003, Poff *et al.* 2003), season (Rosemond 1994, Rosemond *et al.* 2000), and disturbance (Feminella and Resh 1990, Pringle and Hamazaki 1997). For example, a low

standing crop of algae can result from heavy grazing pressure, low light or nutrient concentrations (poor growing conditions), recent disturbance such as a flood, or some combination of these and other factors.

Causal factors responsible for algal abundance patterns are impossible to identify using descriptive or observational approaches alone. Only controlled experiments, those done under field (*in situ*) conditions being best, can be used to evaluate the separate and combined effects of grazing and other factors on producer assemblages. Realize, however, that producers can have reciprocal effects on consumer populations because plants have "primacy" (sensu Power 1992) in many ecosystems. Experiments can be conducted to assess these "bottom-up" effects of plants (e.g., Lamberti and Resh 1983, Lamberti *et al.* 1989), although the emphasis of this chapter will be on grazer effects.

In this chapter, we describe three field experimental approaches to assess the effects of grazers on benthic algal assemblages in streams. All experiments involve the manipulation of grazer abundances over time, using either grazer exclusions or cage enclosures. Optional methods are presented to measure grazer colonization, depletion of algal biomass, and growth of grazers. The specific objectives of this chapter are to (1) provide an introduction to the producers and consumers involved in lotic herbivory; (2) quantify reciprocal interactions between producers and consumers in streams; (3) assess variation in grazing within and among stream sites; and (4) illustrate the advantages and limitations of field experiments for measuring grazer impacts in streams.

These experiments are designed to be conducted in low- to middle-order streams (orders 2–5) where benthic grazers often predominate, but they can be modified for use along the margins of large rivers and even in littoral zones of lakes. Similar experiments can also be conducted within laboratory artificial streams, where a high level of control is possible (reviewed by Lamberti 1993). However, field experiments typically provide more realistic conditions and responses (i.e., higher accuracy) than laboratory experiments. Unfortunately, although more accurate, experiments in natural streams often are prone to more variable responses (i.e., lower precision) than those in the laboratory, and so may require larger numbers of replicates to achieve the same level of precision. Logistical constraints and the possibility of unanticipated events (e.g., floods, drought, vandalism, etc.) also should be considered when designing field experiments. Naturally, in both public and private waterways, permission from appropriate officials or landowners should be obtained before conducting field experiments.

II. GENERAL DESIGN

A. Site Selection

Small to moderate-sized (order 2–5) wadeable streams are preferable for the methods described in this chapter because they typically contain benthic grazers and productive algal assemblages, and often have the high water clarity necessary to allow visual estimates of grazer abundance (see below). If possible, several stream reaches, grouped by contrasting riparian canopy (shading) but all with similar channel form, should be selected. Here, each reach (and its associated habitat units such as pools, runs, or riffles; see Chapter 2) is considered a separate replicate. One group of reaches should have high irradiance (little or no shading) and the other should have low irradiance (heavy shading). In homogenous streams that have little variation in shading, researchers instead may select reaches that differ in current velocity (e.g., low- and high-flow classes) or other factors thought to influence grazing (e.g., streamwater nutrient levels, such as upstream and downstream

of a known nutrient input). Alternatively, simpler, but statistically flawed, experimental designs involve selection of two reaches that differ in some environmental feature or use of a single stream reach. In both cases, reaches are subdivided into replicate habitat units over which experiments are conducted. Use of two reaches and their nonindependent habitat units as spatial replicates to examine effects of selected environmental factors is considered pseudoreplication (sensu Hurlbert 1984) and thus is less desirable than using stream reaches (or even different streams) as replicates. A single stream reach can be used if the study question only concerns grazing and treatments are interspersed (although environmental factors can be measured and used as statistical covariates). These latter two experimental designs may be the only practical approach in some studies of grazing, although they have limited extrapolational power to other streams or stream reaches (Hurlbert 1984).

B. Field Experiments

1. Herbivore Platform Exclusions

Benthic grazers that do not swim or that exhibit low drift rates (e.g., cased caddisflies, snails, etc.) can be excluded by elevating artificial substrata above the stream bottom, which are then mostly inaccessible to those grazers over short periods (Lamberti and Resh 1983, Feminella *et al.* 1989). A "platform" supporting algal substrata (stream rocks or unglazed clay tiles) is erected in each replicate habitat unit and a control plot is placed directly on the streambed adjacent to each platform. Sampling of grazers and periphyton on the treatment and control plots is conducted over time extending to ≥30 d. This design allows comparison of an "ambient" level of grazing (periphyton in control plot) with a "reduced" level of grazing depending on which grazer species are excluded (periphyton in platform plot). Artifacts of caging (see *Basic Method 2*) are minimized with this design, but uncontrolled differences in depth, light, current velocity, or other factors still may exist between each control and platform pair, which may affect experimental results. This bias may be minimized by selecting plots that exhibit minimal environmental variation in all but the variables of interest (Feminella *et al.* 1989). It is also important to note that such designs *rarely* exclude all grazers. Some swimming or drifting nontarget species (e.g., mayflies, chironomids, some fishes), as well as occasional target grazers, may accumulate on platforms, possibly occurring at higher densities than on the stream bed (e.g., Lamberti *et al.* 1992). These animals will require manual removal periodically during the experiment. If this is not feasible, it may be necessary to estimate the abundance of these grazers during the experiment and consider it as a covariate in statistical analyses.

2. Herbivore Cage Enclosures

Alternatively, grazer species and density can be manipulated directly within stream enclosures or "cages." These can be made from simple materials, stocked with known densities of grazers, and can be submersed (e.g., Feminella and Hawkins 1994, Stelzer and Lamberti 1999) or floated in the stream (e.g., Lamberti *et al.* 1987b). One set of cages is deployed in each stream section (e.g., shaded vs. open reaches, high- vs. low-current microhabitats, etc.) and sampling of all treatments is conducted over time extending to ≥30 d. This design allows comparisons among known levels of grazing, which can range from zero to high, while exerting more control over grazing pressure than provided by

the platform design. It is also possible to measure grazing by particular species and/or size classes, which may exert very different impacts on periphyton (Feminella and Resh 1991, Steinman 1991). However, some consideration must be given to potential cage effects that result from altered (usually reduced) flow, increased sedimentation, and colonization by unwanted grazers (see Walde and Davies 1984, Cooper *et al.* 1990); each of these may alter the effectiveness of grazer manipulations and confound interpretation of results. In addition, more maintenance generally will be required for cages than for platforms. In some instances, however, such as when conducting intraspecific grazing experiments or when quantifying effects of individual grazers on periphyton, cages may be the only suitable design.

3. Electric Exclosures to Manipulate Consumers

An *in situ* experimental technique using electricity was developed to meet some of the challenges of working in dynamic stream environments that are characterized by unidirectional current and discharge fluctuations (Pringle and Blake 1994, Pringle and Hamazaki 1997). This electric exclosure technique allows manipulation of stream macro-consumers (e.g., fishes, amphibians, macrocrustaceans) under realistic hydrodynamic conditions. The technique does not result in reduction of stream current and increased sedimentation, as can occur in caging experiments. Electrified plots (small squares or hoops placed over replicated areas of the stream bottom) are wired to solar or battery-powered (6–12 volt) fence chargers (mounted on the stream bank) that emit continuous electric pulses to repel macrobiota. This technique has been used to examine the effects of fishes (Pringle and Hamazaki 1997, 1998), shrimps (Pringle and Blake 1994, March *et al.* 2002), and larval anurans/tadpoles (Ranvestel *et al.* 2004) on algal and macroinvertebrate communities. Pringle and Hamazaki (1998) used electric exclusion to manipulate the presence of diurnal fishes and nocturnal shrimps by turning the electricity on and off during day- and night-time hours, depending on the desired treatment. Their findings showed that fishes and shrimps separately exerted strong direct trophic effects, which resulted in cumulative effects on benthic algal standing crop and community composition in addition to affecting benthic invertebrate communities.

Whereas most field-based, experimental studies of stream grazing have been conducted during, or were designed to simulate, baseflow conditions (see Feminella and Hawkins 1995), data on how trophic forces interact with abiotic disturbance in streams, such as high discharge, are lacking because severe physical conditions impose logistic constraints on *in situ* experiments (e.g., floods can destroy cages). An advantage of the electric exclosure technique is that it allows for examination of top-down trophic effects on algae under natural hydrologic conditions including discharge fluctuations. Effects on algae can be assessed in a relatively natural depositional environment subject to natural background erosion and sloughing. For example, Pringle and Hamazaki (1997) examined how natural fish assemblages interacted with high-discharge events *in situ* (160-fold increases in discharge over base flow) and found that fishes played a key role in maintaining the stability of benthic algal assemblages and their resistance to storm events. This technique also has allowed evaluation of increases in stream bedload on top-down interactions of macroconsumers in both algal- and detrital-based streams (Schofield *et al.* 2004). Results indicate that small, yet environmentally realistic, increases in bedload affect benthic communities, primarily by alteration of fish effects.

Electric exclosures also have been used effectively to assess top-down effects of macroconsumers on rates of *detrital processing* by measuring rates of leaf pack decomposition

in the presence and absence of fishes (Rosemond *et al.* 1998), crayfishes (Schofield *et al.* 2000), and shrimps (March *et al.* 2001). Whereas all of these studies have manipulated the presence and absence of macroconsumers, it is also possible to exclude smaller organisms such as aquatic insects using electricity. Biota are affected by electric fields in proportion to their body size (i.e., large organisms are affected more strongly than small organisms). Brown *et al.* (2000) used high-powered electric fence chargers to inhibit the grazing of mayflies (Ephemeroptera) in an Australian stream, as aquatic insect exclusion requires more powerful (higher voltage) chargers and/or shorter distances between electrodes. Although mayflies were not totally excluded by these high-powered electric exclosures, their grazing activity was reduced by the treatment, as evidenced by increases in algal standing crop. In an experiment employing two intensities of electrical current, Moulton *et al.* (2004) excluded both shrimps and mayflies (high-intensity electric treatment) or only shrimps (low-intensity electric treatment) from benthic areas in a coastal neotropical Brazilian stream. Last, Taylor *et al.* (2002) used a reach-scale electroshocking technique in a Colorado stream to reduce densities of grazing invertebrates by 86%, which resulted in significant increases in algal standing crops.

C. Optional Methods

The above methods are designed primarily to determine the effects of consumers on benthic producers. However, these approaches can be expanded to assess specific effects of producers on consumer populations as well. For example, the platforms can be used do "culture" algae in a grazer-free condition, and then after returning those periphyton patches to the stream bottom, the rate of grazer colonization and depletion of those patches can be determined (e.g., Lamberti and Resh 1983). The cage method can be used to measure grazer growth rates by determining starting and ending weights of grazers, which can test for grazer competition (e.g., Lamberti *et al.* 1987b) or facilitation among consumers (Feminella and Resh 1991, Heard and Buchanan 2004). Electric exclosures can accumulate uneaten detritus that then provides resource 'islands' for other consumers unaffected by the electricity.

Grazer manipulations in streams are not limited to the three approaches described above; certain types of grazers can be manipulated effectively using other techniques. For example, densities of certain sedentary or sessile grazers (e.g., fifth-instar hydroptilid caddisflies) can be altered by direct removal of animals from rock surfaces (e.g., McAuliffe 1984, Hart 1985). In another approach, insecticides can be mixed with agar in diffusing substrata to deter some grazers (e.g., chironomid larvae) from colonizing those substrata (Gibeau and Miller 1989, Peterson *et al.* 1993). However, care must be taken when using insecticides to avoid deleterious effects on nontarget organisms.

D. Laboratory Analyses

In the preceding methods, periphyton is sampled and analyzed for biomass and chlorophyll *a* content at the end of the experiment (see methods in Chapter 17), although many other variables also can be measured if desired. In *Basic Method 1* below, macroin-vertebrate community structure and density are determined for control (grazed) and platform (ungrazed) plots. For *Basic Method 2*, growth rates of grazers in enclosures are determined by weighing grazers at the beginning and end of the experimental period. In the *Advanced Method* using electric exclosures, we demonstrate how other ecosystem

processes (e.g., decomposition, bioturbation, trophic cascades) can also be studied during the experiment.

III. SPECIFIC METHODS

A. Basic Method 1: Grazer Exclusion Using Platforms

1. Platform Construction

1. Construct 5–10 grazer-exclusion platforms, the exact number depending on the experimental design (see below). Platforms are made of J-shaped aluminum or steel supporting rods and square 1/4″ Plexiglas® plates, the latter of which are used to hold tiles or rocks as a periphyton substratum. The resulting platform should look similar to the ones shown in a stream in Figure 23.1. It is also possible to make platform supports from polyvinylchloride (PVC) pipe and elbow joints, depending on how rigorous the stream environment may be.

2. Cut Plexiglas® into ~400 cm^2 plates (approximate dimensions = 20 × 20 × 0.4 cm). Drill a 3/8″ hole midway through one of the four sides, about 1 cm from the edge, which allows the plate to be attached to the supporting rod. Cut a second set of plates for use in the control plots; these do not require a hole.

3. Using a drill press or metal lathe to secure the supporting rod, drill a 5/16″ hole (ca. 1″ long) into one end of the rod. Next, using a 3/8″ tap, thread the drilled hole to accept a 3/8″ hex bolt. Bend the threaded rods into the necessary J-shape with a metal jig in a bench vise, with the threaded end on the shorter end of the bend. (This is most easily done with aluminum rods; steel rods are more durable but required heating before bending, which should be done by a qualified machinist.) Screw a hex bolt into the hole to ensure that it is seated properly.

2. Initial Fieldwork

1. Before embarking on a field study, give thought to the scale of the grazing question you are asking and then design your experiment appropriately. Regardless of whether you are working with platforms, cages, or electric fences, you should consider that one unit (platform, cage, or fence) is equal to one experimental replicate (Hurlbert 1984). Therefore, to achieve a treatment n=3, for example, you will need three platforms, cages, or fences and an equivalent number of control units for comparison. Any sample taken from one of those units at a particular time contributes only one replicate; additional samples from that same unit constitute only "subsamples." Samples taken over time from the same experimental unit are not true replicates but rather are "repeated measures" (Zar 1999). Decide at what spatial scale you wish to ask the grazing question—within a channel unit (e.g., pool), along a stream reach, or over a valley segment (see Chapter 2). Match the distribution of your replicates to this "inference space." Place your replicates (typically a paired treatment and control plot) at random within the channel unit of interest, along the study reach, or dispersed over a segment. Measure other environmental covariables that might influence the outcome of the experiment, such as canopy cover, current velocity, and water depth. Analyze the results using an appropriate statistical test (see IIID below).
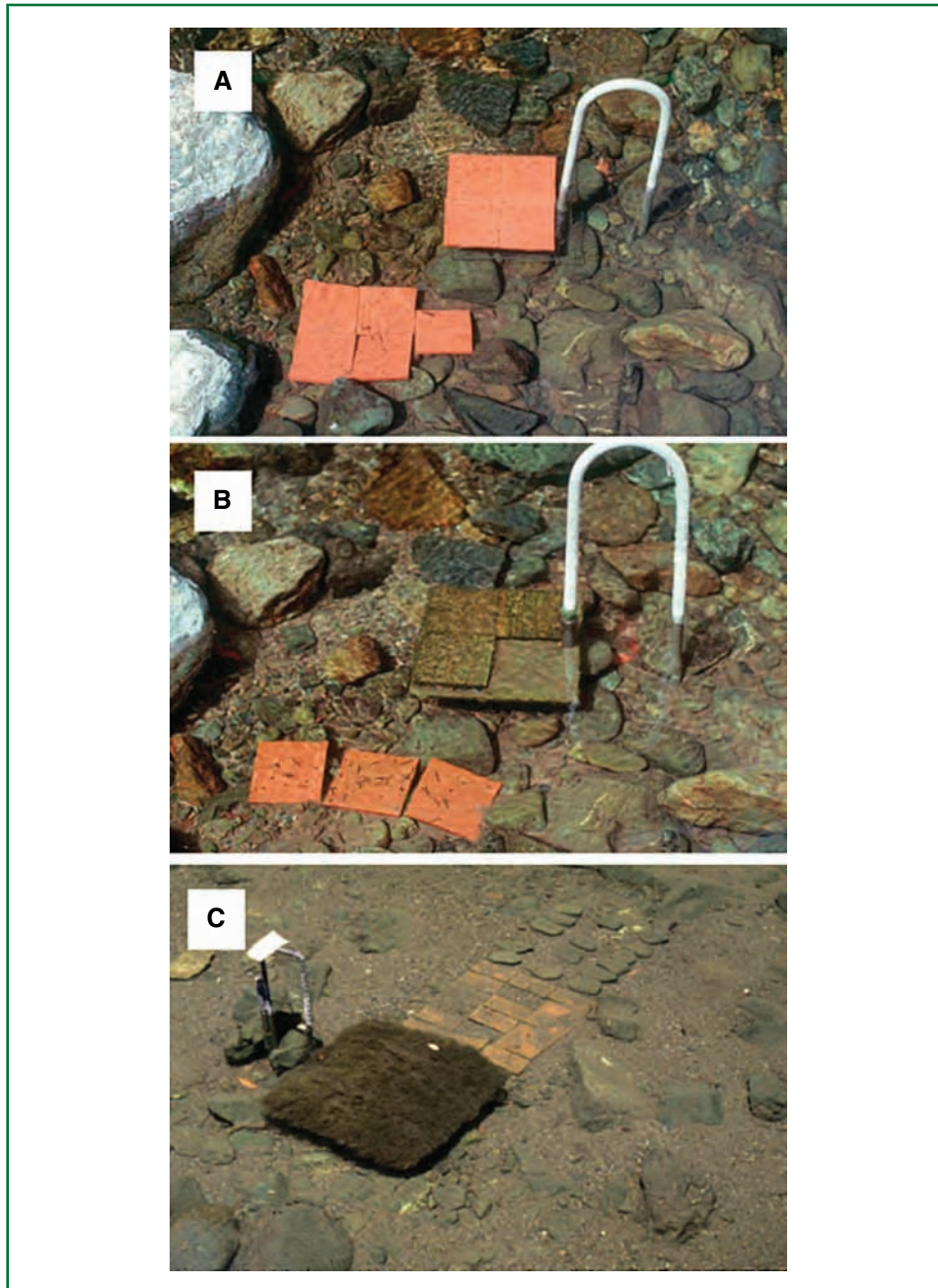
FIGURE 23.1 Grazer exclusion using platform design. Aluminum platform design showing quarry tiles (7.5 × 7.5 cm) on raised plexiglas plates (ungrazed platforms) and on the streambed (grazed controls) at the beginning (A) and end (B) of the experiment. Tiles missing from platform and control plots in (B) were sampled before photograph was taken and objects on control tiles are grazing caddisflies (from Feminella *et al.* 1989). (C) Metal platform design showing periphyton growth at the end of a 90-d grazer exclusion in a California, USA, stream. Note luxuriant algal growth on the platform; rocks at upper right were used to calibrate clay tiles (see Lamberti and Resh 1983).

2. Incubate unglazed clay tiles on the streambed for at least two weeks (and preferably for one month) to allow algal colonization; the number of tiles incubated should be enough to supply all plots plus an additional 10% to allow for loss. (Sterilized rocks, such as those obtained from a quarry, can be substituted for tiles to provide more realism in any study that we describe. From here forward, however, we will describe methods for the use of tiles.) A tile size of 7.5×7.5 cm is appropriate, four of which can be cut from one standard $6'' \times 6''$ tile using a tile cutter or masonry saw. Tiles should be incubated in a single microhabitat (similar depth, flow, and shading) so that similar periphyton assemblages are present on all tiles across all treatments at the beginning of experiments.

3. Installation of Platforms

1. Install supporting rods for platform plates in the selected sites within the stream. We recommend use of reinforcement bar (of similar diameter to that of supporting rods) and a small sledgehammer to make a pilot hole in the substratum. Once embedded into the streambed about 30 cm, carefully remove the pilot bar and in its place insert the supporting rod; the rod is then tapped in place with the sledge. Next, secure four precolonized tiles to each platform and control plate with dabs of silicon sealant on the undersides or with two heavy-duty rubber bands; use of silicon will prevent accidental dislodgement of tiles during the experiment. If rubber bands are used, be sure to subtract the area covered by rubber bands on each tile when estimating periphyton abundance later, as tile areas underneath rubber bands will not accrue algae. Attach the platform plate to the supporting rod with a washer and hex bolt; use a wrench to tighten firmly but do not overtighten or plate may break. Place the control plot (plate with secured tiles accessible to grazers) on the stream bed near the platform but not shaded by it.

2. At each location, install one replicate platform and one control plot, while matching current velocities, shading, and, if possible, depth. The latter may be achieved by using natural streambed contours to minimize the platform distance above the substratum, while maintaining sufficient elevation to prevent colonization by crawling grazers. Alternatively, in more homogenous-bottomed streams, bricks or cinder blocks may be used underneath control plates, which serve to match elevation (depth) with paired platforms, yet still permit grazer access to tiles (Feminella *et al.* 1989). It is best to place each platform and control plot side-by-side (rather than upstream-downstream) so they will have minimal flow influences on each other.

3. Measure physical parameters for each platform and control plot including water depth, current velocity, irradiance, and nutrient levels (if relevant; see Chapters 9–11). Current velocity over the substrata can be measured with a current meter or by releasing inert dye (e.g., fluorescein) with a pipette and measuring time-of-travel (see Chapter 4). Irradiance can be measured with a portable light meter held close to the water surface (or with an underwater probe; see Chapter 5), or estimated indirectly by measuring overhead canopy with a fish-eye camera lens or spherical densiometer (Lemmon 1957, Feminella *et al.* 1989). One set of four canopy measurements (i.e., facing upstream, downstream, and right and left banks) can be taken for both platform and control plot at each site; the four readings are then averaged for a single estimate for that site.

4. Sampling Platform Substrates

1. Decide on how many tiles to sample from each plot (platform or control) at the end of the experiment (≥ 30 d). Because each plot represents one replicate, it is necessary to sample only one tile per platform and control couplet. However, to reduce variability, you may want to sample two or more tiles per plot and then pool the sampled material before analysis. The same tiles can be used to sample both macroinvertebrates and periphyton. However, extra tiles may be needed for additional analyses such as algal taxonomic composition, primary production, or elemental composition. Sampling over time (e.g., one-week intervals) also can be employed to examine how patterns develop over time (e.g., Lamberti and Resh 1983).

2. Sample macroinvertebrates from tiles at the end of the experiment. Dislodge macroinvertebrates from a tile into a downstream net (mesh size $\leq 250\,\mu$m) and then empty the net contents into a labeled heavy-duty plastic sample bag (e.g., Ziploc® or Whirl-pak®) or jar and preserve with 70% ethanol. Be sure to sample undersides of tiles for mobile invertebrates (e.g., mayflies, stoneflies, etc.). Manually remove any sessile invertebrates such as black flies, chironomid midges, and caddisflies. The latter two groups often attach their organic (algal-rich) cases to tiles, which may remain after larvae are removed. Thus, investigators should indicate whether they removed cases prior to sampling or left them in place as part of the periphyton sample.

3. Sample periphyton from tiles after invertebrates have been removed. In the field, periphyton can be scraped or brushed from the tile into water and placed in a darkened container on ice. Alternatively, the entire tile can be placed in a plastic container, stored on ice in the dark, and scraped in the laboratory later the same day. Analyze periphyton for biomass (as ash-free dry mass, AFDM) and chlorophyll *a* content and also compute the biomass/chlorophyll *a* ratio (see Chapter 17). *Optional:* determine algal taxonomic structure (see Chapter 16), primary production (see Chapter 28), or elemental composition (e.g., Stelzer and Lamberti 2002; see also Aberle *et al.* 2005).

B. Basic Method 2: Grazer Manipulation Using Enclosures

1. Enclosure Construction

1. Instream enclosures can be constructed from various materials, or prefabricated containers can be modified for use. All designs should be fitted with mesh on the upstream and downstream sides to allow for water exchange. Enclosure (and mesh) size should be scaled to the size and density of grazers used in the study. Animals vary greatly in their ability to behave "normally" within an enclosure, so there is no steadfast rule governing the size of the cage relative to the size of the animal used. However, the best choice in a cage size is one that collectively (1) provides the best possible control of the variable of interest; (2) is economically and logistically feasible to build, deploy, and maintain; and (3) has the lowest potential for cage-related artifacts.

2. Construct cages (n = 20–30) suitable for the grazer of interest. For small grazers (e.g., small caddisflies or snails), plastic food containers with two or more sides replaced by window screening may be suitable (Figure 23.2A). Cut window screening (1-mm mesh) to size and secure to containers with silicone aquarium sealant or chemically inert hot glue. Lightly roughen container edges with sandpaper prior to attaching the screen to maintain the adhesive bond for a longer
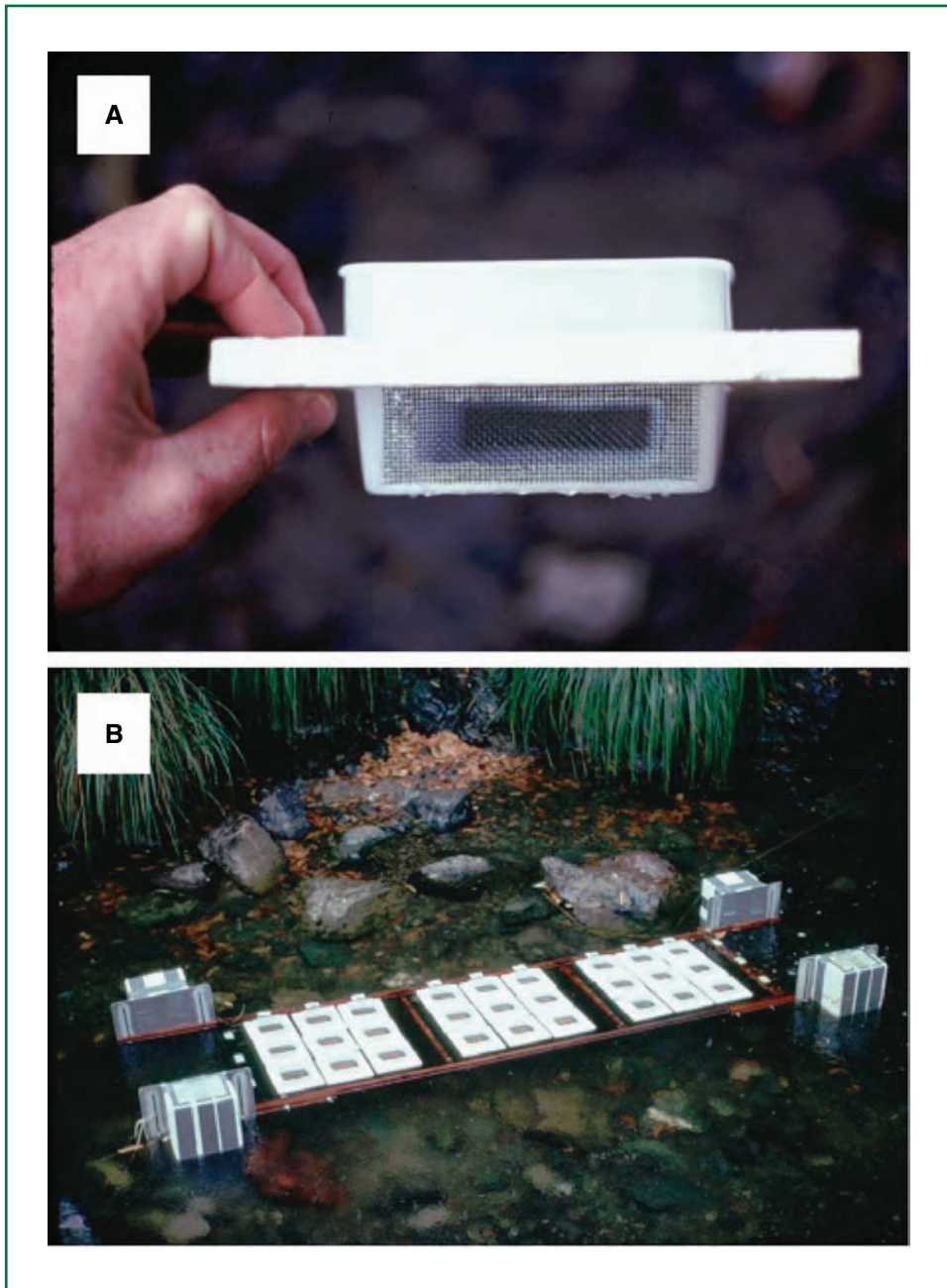
FIGURE 23.2 Grazer enclosures used to manipulate densities of small benthic grazers. (A) Each enclosure is ~500 cm^3 with sides screened with 1-mm^2 mesh and fitted with a styrofoam collar for flotation. (B) Floating rack holding 27 enclosures tethered in a stream run (from Lamberti *et al.* 1987b).

period. Allow at least 24 hours for the adhesive to cure before immersing the enclosure. For large grazers (e.g., limnephilid caddisflies, crayfish, tadpoles, small fish), larger enclosures made of hardware cloth, porous plastic containers, plastic gutters, PVC pipe cut longitudinally (Figure 23.3; see Feminella and Hawkins 1994), or other inexpensive materials may be used. These larger, more durable materials also allow experiments to be performed in fast-flowing stream sections, such as in riffles or runs, where rheophilic grazers (e.g., heptageniid mayflies, glossosomatid caddisflies) predominate. It is important to note, however, that use of galvanized metal hardware cloth for enclosures, even when used *in situ*, may have toxic effects on caged grazers; thus, use of inert plastic or fiberglass mesh rather than metal for enclosures is strongly advised.

3. Enclosures may float or be submerged. To float small enclosures at the stream surface, cut "collars" from 1/2″–3/4″ thick styrofoam panels (Figure 23.2A). Leave enough of a styrofoam border around the complete enclosure so that it remains buoyant and stable when placed in the stream. Covers for individual enclosures (as in Figure 23.3) can be used if there is a need to confine large stream animals or reduce predation by terrestrial animals, although they are not recommended in grazing studies if they modify irradiance. The enclosure may contain a substratum tile, grazers, and additional materials, such as sand for caddisfly case-building material. Sets of enclosures may be held in place within rectangular wooden racks, which are pre-drilled and strung tennis racquet-style with monofilament line (30–60 lb test) to secure individual enclosures and collars (Figure 23.2B). Racks can be floated by attaching styrofoam blocks to each of the corners and held in place by

FIGURE 23.3 Photograph of 12 large, submersed PVC enclosures (dimensions: 103 cm L × 32.5 cm D) used to study grazing tadpoles in high-gradient streams near Mount St. Helens, Washington, USA. Enclosures contain algal-covered stream cobbles that are used both as grazing substrata for tadpoles and as ballasts for enclosures in fast riffles. Coarse mesh covers used to isolate and protect animals are shown in the 6 enclosures in the upper part of photograph. Direction of flow is from upper left to lower right of photograph (see Feminella and Hawkins 1994).

tethering to rebar stakes or trees on the bank. Some slack in the tethers is desirable in case water level changes. A wooden frame fitted with a chicken-wire screen and placed over the rack may protect enclosures from falling debris or disturbance from small animals. Alternatively, individual enclosures can be tethered to stakes or bricks in the channel with monofilament, and allowed to "free float" in the stream. The ideal location for racks is in a stream "run," which provides both flow and flotation room.

4. Submersed enclosures are placed directly on the stream bed. In swift current, it may be necessary to attach them with hose clamps, twist ties, or other materials to reinforcement bar pounded into the stream bed or to concrete cinder blocks (Figure 23.3). For larger enclosures, stream cobbles lining enclosure bottoms may be used as ballasts. Unless enclosure walls are considerably higher than that of the stream surface, enclosures must be completely covered with mesh so that grazers are effectively isolated and experimental treatments can be maintained.

2. Initial Fieldwork

1. Locate a study site that will receive enclosures. It is less critical to replicate the experiment over several sites (cf. platform design), because a full range of experimental treatments and replicates can be interspersed within a single site and inference across a larger space is less important. In this enclosure study, the main effect is grazer density and the response consists of various periphyton variables.

2. Incubate unglazed clay tiles on the stream bed, as described previously (Basic Method 1—Initial Fieldwork).

3. Installation of Enclosures

1. Survey the stream reach to identify the numerically dominant large, benthic grazer(s) in the stream (e.g., snails, caddisflies, crayfish, etc.). Measure the ambient density of these grazers on the stream bed by making visual counts from replicate quadrats (N = 15–30) placed randomly in all habitat types (e.g., riffles and pools) throughout the study reach, as well as in specific microhabitats where grazers are most common (e.g., cobbles, boulders). These two separate estimates will yield a full range of grazer densities from low (across all habitat units) to high (within preferred habitat); doing so will yield a large operational range of grazer abundances in which to bracket experimental treatments and will provide greater extrapolational power for experimental results. Quadrat size may be scaled to the size, density, and spatial distribution of the grazer studied. For example, small quadrats ($<100\,cm^2$) may be precise enough to characterize small, abundant grazers such as mayflies, sessile midges, or small caddisflies, whereas much larger quadrats ($\gg 100\,cm^2$) may be required for larger, more patchily distributed grazers such as crayfish, tadpoles, or large caddisflies.[1]

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[1] Visual counts are generally only useful in estimating densities of large, slow-moving grazers that are easily seen on the streambed surface. This approach may not be feasible in streams with high turbidity (low water clarity) or for grazers that cannot be quantified visually, such as those species occurring in subsurface habitats or exhibiting low activity (i.e., visibility) during daylight hours. In these cases, direct substratum sampling (e.g., Surber or Hess samplers) or nighttime estimates (Feminella and Hawkins 1994) may be more appropriate (see Chapters 20–21 and Resh *et al.* 1984).

2. Choose treatments and number of replicates for each treatment. Use ambient grazer density and no grazers, with three replicates of each treatment. Optionally, include treatments of one-half ambient density and double ambient density, or add replicates to increase statistical power. In a more complex design, shading or current velocity contrasts can be added to some replicates to achieve a two-factor experimental manipulation (i.e., grazing and irradiance/current). Shading can be achieved by placing neutral-density shade-screen over the top of some enclosures, whereas velocity contrasts can be achieved by placing the full complement of density treatments within replicated stream sections with the desired velocity classes (e.g., 0–4 cm/s [low], 10–15 cm/s [moderate], and/or ≥30 cm/s [high velocity]).

3. Select a relatively uniform site in the study reach in which to install enclosures. Place precolonized tiles in enclosures and suspend in the rack or anchor to the stream bottom (see above). Collect individuals of the selected grazers from the stream. Randomize the collected grazers and place the appropriate number of animals into each replicate enclosure.[2] Establish a block in each identified microhabitat (velocity range, depth, etc.) and place one replicate of each treatment in each block. Ideally, the number of blocks should correspond to the number of treatments so that treatments are equally represented across blocks.

4. Sampling Enclosures

1. At the end of the experiment, sample all enclosures containing tiles and grazers; the same tiles can be used to quantify both macroinvertebrates and periphyton abundance. However, extra replicate enclosures may be needed for additional analyses such as algal taxonomic composition, primary production, or elemental composition.

2. Manually remove grazers and all other macroinvertebrates from enclosures at the end of the experiment (≥30 d) and preserve all in 70% ethanol or 5% formalin.[3] Count the remaining target grazers; identify and count other macroinvertebrates that have colonized the enclosures. Quantify the difference between starting and ending grazer abundance to determine mortality. Optionally, measure grazer growth (see below).

3. Sample periphyton chlorophyll *a* and biomass from tiles at the end of the experiment, as described previously and in more detail in Chapter 17.

4. *Optional*: Estimate growth rates of grazers in enclosures to determine if growth is density-dependent. If target grazers are approximately the same size (e.g., synchronously developing aquatic insect cohort), then subsample animals at the beginning of the experiment to determine average starting individual mass as tissue dry mass or AFDM (see Chapters 17, 20). At the end of the experiment, remove and preserve grazers from enclosures, and estimate individual grazer mass (determined individually or batch-weighed) for each replicate enclosure. Determine also individual mass of grazers from the stream bed, collected on the same day as those from enclosures; this step will allow an assessment of grazer growth patterns

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[2] Minimize position effects (e.g., velocity or depth gradients) by using a randomized complete block design (see Zar 1999).
[3] Formalin is preferable to ethanol for preserving biomass because less tissue is lost to leaching. However, formalin is a known carcinogen and therefore extreme caution should be exercised in its use.

attributable to enclosures themselves (i.e., cage effects). If cage effects are minimal, growth of grazers in enclosures containing "ambient" densities will be equivalent to those on the stream bed. If grazers are slow-growing or occur as multiple cohorts, then growth of tagged individuals will need to be determined. We have used individually numbered "bee" tags, glued carefully to the shells of snails, cases of caddisflies, or carapace of crayfish to monitor individual growth (e.g., Lamberti *et al.* 1989).

C. Advanced Method: Consumer Manipulation Using Electric Exclosures

1. Electric Exclosure Construction

1. Construct 10 PVC frames (0.25 m^2) lined with copper wire. Each square frame is constructed of four lengths of 0.5-m long PVC frame and corner connectors fitted with two concentric squares of uninsulated 12-gauge copper wire (Figure 23.4). The outer and inner squares of copper wire are held to the PVC tubing with plastic cable ties. All 10 frames should be constructed in the same manner, with the difference between the five control and five exclusion treatment frames is that exclusions are connected to solar- or battery-powered fence chargers (electrified treatments), whereas controls are not. The resulting frame should resemble the one shown in Figure 23.4.
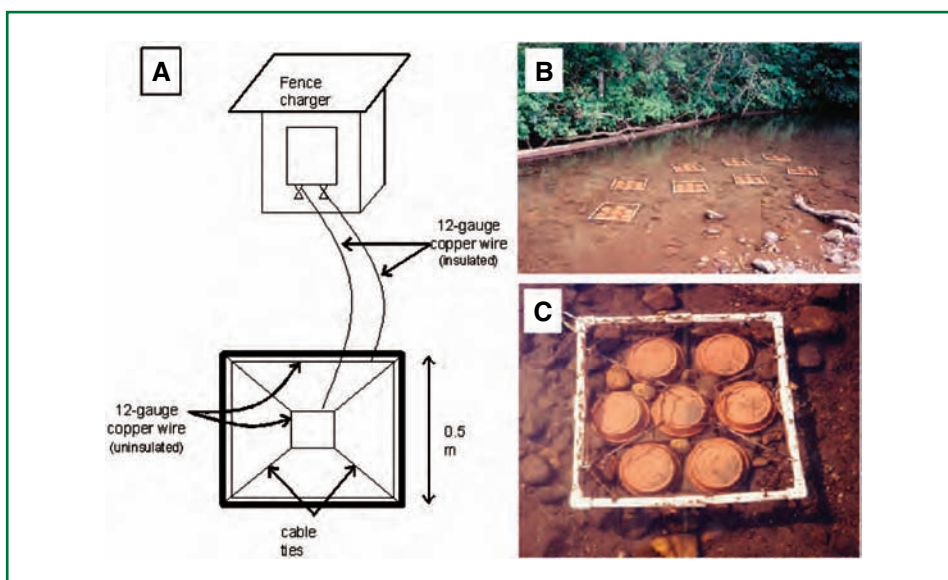
FIGURE 23.4 Electric exclosures in the field. (A) Schematic diagram of one electric exclosure showing basic wiring plan; (B) Block of 8 frames (4 electric and four control) installed on the bottom of a North Carolina, stream. Four of the frames are hooked up to solar-powered fence chargers (not visible and installed in the sun higher on the bank), while four of the frames are not electrified; (C) Close-up of one electric exclosure frame installed on the stream bottom and containing seven nutrient-diffusing clay saucers (from Dye 2005).

2. Initial Fieldwork

1. In this study, the main effect is macroconsumer presence or absence and the response variable is algal standing crop (e.g., chlorophyll *a*, AFDM; see Chapter 17) and algal assemblage composition (see Chapter 16). We suggest that benthic insects also be included as a response variable (see Chapter 20). It is possible to run this same experiment using rates of leaf decomposition as the response variable (see Chapter 30), substituting 5-g (dry mass) leaf packs for clay tiles and retrieving leaf packs from treatments over time (see Rosemond *et al.* 1998, March *et al.* 2001, or Schofield *et al.* 2001 for modifications of leaf breakdown experiments using electric exclosures).

2. Before running an experiment, the effectiveness of fence chargers to exclude macrobiota must be directly tested within the study stream, as the strength of the electric charge of a given model of fence charger varies with water chemistry (i.e., conductivity), size of the organism, and whether organisms are repelled by electric pulses emitted by the charger. A 6-volt fence charger has been shown to be effective at excluding all fishes in study streams in lowland Costa Rica (Pringle and Hamazaki 1997) but may not be effective in other streams with lower conductivities. For example, 6-volt chargers completely excluded fishes in southern Appalachian streams of North Carolina and lowland streams of Costa Rica, but 12-volt chargers were most effective in lower conductivity waters in Panama (Ranvestel *et al.* 2004).

3. The experimental design will involve establishing five experimental blocks within a representative 100-m stream reach. Each block will include one electric exclosure and one adjacent control treatment frame, with locations of blocks occurring within standardized stream conditions (i.e., similar current velocity, depth, canopy cover, etc.) depending on the type of algal/grazer assemblage being examined and the macroconsumer being manipulated. For example, in a study examining effects of grazing tadpoles on algae and benthic insects, Ranvestel *et al.* (2004) established blocks in runs because grazing tadpoles were abundant in this habitat type and water depth and velocity were less variable there than in riffles or pools.

4. As recommended in previous methods within this chapter, the experimental duration of the experiment should be 30 d to allow establishment of algal assemblages. At least six unglazed clay tiles should be placed within each treatment frame, with one tile retrieved from each frame every 5 d over the experiment.

3. Installation of Experiment

1. After placement of one control and one electric exclosure treatment frame within similar physical conditions in each of five blocks (described above), each of the frames should be flush with the stream bottom and anchored in place using devices appropriate to the bottom substratum. For example, Schofield *et al.* (2004) affixed frames to metal spikes driven into the cobble bottom of a southern Appalachian USA stream, whereas Pringle *et al.* (1999) affixed frames onto flat bedrock surfaces using underwater epoxy in Puerto Rican streams. Each electric exclusion replicate should be connected to a *fence charger* by attaching appropriate lengths of insulated 12-gauge copper wire from the inner square to the *power source* and from the outer square to the *ground*. Frames should be placed to minimize any influence of macroconsumer exclusion treatments on adjacent control treatments (0.5-m gaps between adjacent frames usually will suffice).

2. Tether six unglazed ceramic tiles (7.5×15 cm) with cable ties and binder clips to the PVC within each experimental frame.
3. Monitor the experiment on a daily basis to (a) ensure that fence chargers continuously emit pulses of electricity and to replace batteries when necessary (it may be necessary to change batteries every 3–5 d to maintain a strong, consistent electric charge) and (b) to remove any debris (leaves, twigs, etc.) that may have become caught on copper wire or PVC frames; such debris can alter current velocity, shade, and thus trophic dynamics.

4. Experimental Sampling

1. Retrieve one tile from each frame every 5 d for analyses of algal standing crop and assemblage composition through time. Tile retrieval will consist of cutting cable ties and removing the tile from the water within a fine-mesh hand net to prevent loss of invertebrates. Care should be taken not to disturb any sediment that may have accrued on tiles. Tiles and the contents of the hand net should be immediately placed into Ziploc® and transported to the laboratory in a cooler.
2. In the laboratory, the top surface of tiles should be scraped with a razor blade and scrubbed thoroughly with a toothbrush to remove algae. Once invertebrates are removed (and preserved in 70% ethanol for later identification), the homogenate of sediment, algal periphyton, and other fine particulates should be subsampled for chlorophyll *a*, AFDM, and algal species composition (and algal biovolume, if desired; see Chapter 16).
3. To identify those macrobiota that visit the control treatment frames (but are repulsed by the electrified plots), establish a schedule of systematic observations. For example, to identify fishes and shrimp taxa that were foraging on control tiles in an electric exclosure experiment within a Puerto Rico stream, March *et al.* (2002) conducted 90-sec observations of each treatment replicate on several dates during both the day and night.

D. Data Analysis

1. Exclosure Experiments (Platforms or Electric Exclosures)

Parametric statistics can be used to analyze data, provided that assumptions of normality, homogeneity of variance, and sample unit independence are met (Zar 1999). For the platform or electric exclosure studies in which there are two grazing levels (i.e., platform vs. control plots), an independent *t*-test (or one-way ANOVA with two groups) can be used to compare the treatments. An even better approach may be a paired *t*-test (parametric test) or Wilcoxon signed-rank test (nonparametric test). These tests examine the *difference* between the paired control and platform plots for a specific response variable and will statistically remove the variation associated with unaccountable differences among the paired plots. Differences between paired platform and control plots can then be compared for each variable (both treatment and response) measured (e.g., periphyton AFDM, chlorophyll *a*, grazer density, etc.). If samples are taken over time, then a repeated-measures ANOVA can be used to compare treatment and control responses over the experiment (Zar 1999). The latter analysis will allow a simultaneous assessment of (1) treatment effects (e.g., effects of grazers on periphyton measures), (2) time effects (e.g., periphyton measures varying over the experiment, and (3) the treatment × time

interaction (e.g., effects of grazers on periphyton measures varying over time). Add covariables to the statistical model as needed.

2. Enclosure Experiments

If only grazing (≥ 2 levels) was manipulated, then a one-way ANOVA or its non-parametric equivalent (Kruskal-Wallis test) is appropriate (Sokal and Rohlf 1995). If, in addition to grazing, a second factor (e.g., irradiance) is manipulated, then a two-factor ANOVA should be used or a nonparametric equivalent (Friedman test). If ANOVA reveals significant differences among treatments, *a posteriori* multiple contrasts (e.g., Tukey HSD test) can be used to identify where specific differences reside (Zar 1999).

3. Regression Approaches

As an alternative to the above analyses, simple regression can be used to examine the relationship between grazer density, light, or current (independent variables) and periphyton abundance or grazer growth (dependent variable). This analysis may be appropriate if densities or abiotic factors vary continuously across the full range of treatment replicates or change substantially over time for individual replicates. Construct graphs of treatment-specific grazer density, irradiance, or current (on x-axis) against corresponding periphyton abundance or grazer growth (on y-axis), and choose the regression model (e.g., linear, exponential, power, etc.) that best accounts for the highest amount of variation in the data (Sokal and Rohlf 1995). If grazer density was the only "fixed" factor in experiments, then it may be most appropriate to treat measured environmental variables as covariates in the analyses (Zar 1999). Multiple regression also be may used to examine collectively the predictive power of all independent variables on periphyton abundance.

IV. QUESTIONS

1. Were the exclosures (platforms or electric exclosures) effective at eliminating some or all consumers? Did you notice any colonization by invertebrates other than the ones you were trying to exclude? Were there any confounding effects of the exclosure?

2. Were there significant effects of consumers on producer abundance (or other measured response variables)? If tested, were there significant effects of canopy or current velocity on periphyton within or among grazed treatments (i.e., grazer × canopy/current interactions)? What can you conclude about the relative importance of light, current, and grazing on periphyton abundance in your stream(s)?

3. If the consumer exclusion resulted in significant periphyton accrual, what does this indicate about the overall importance of grazing in your stream(s)? Can you conclude that all of the stream bed would accumulate that much periphyton in the absence (or reduction) of grazing? Why or why not? Would you expect this same result during all seasons or in all other streams within or outside the watershed/region?

4. If multiple grazer treatments were used in enclosures, what was the relationship between consumer density and producer abundance? If grazer growth was measured, how did this vary with density, and what can you conclude about the importance of intraspecific and/or interspecific interactions among grazers?

5. Did the biomass/chlorophyll *a* ratio differ among grazing treatments? Among stream reaches? Why might this pattern occur and what does it indicate?
6. Do you believe that periphyton is a limiting resource for grazers in your stream? Why? Is it possible for the biomass of benthic grazers to exceed that of primary producers? How might this occur? If producers were indeed limiting and consumers were competing for this resource, how might you test for the specific competitive mechanism (i.e., exploitation vs. interference)?

V. MATERIALS AND SUPPLIES

Platform Materials

> 1/4″ plexiglas squares (20×20 cm)
> 1/2″ aluminum bar
> 3/8″ drill bit, drill, and drill press (or metal lathe)
> 3/8″ hex bolts (1/2″ long), washers, and nuts
> 3/8″ tap for hex bolt
> Form or jig used to bend aluminum bar into J-shape
> Heavy gauge rubber bands
> Reinforcement bar (used for pilot hole)
> Sledgehammer
> Unglazed clay tiles (7.5×7.5 cm)

Cage Enclosure Materials

> Aquarium (silicone) sealant
> Duct tape
> Fiberglass mosquito netting ($\sim$1-mm mesh size)
> Monofilament nylon (30 to 60-pound test)
> Plastic food containers (pint size or larger) or similar enclosure
> Queen bee tags
> Reinforcement bar
> Rope for rack tethers
> Styrofoam blocks for rack flotation ($\sim$1 ft^3); 4 blocks per rack
> Styrofoam sheets to make collars to float enclosures within rack; one per enclosure
> Utility knife
> Wood frame and hardware (for floating rack), large enough to fit all enclosures

Electric Exclosure Materials

> Polyvinyl chloride (PVC) pipe to make square frames (40 0.5-m lengths; 2.5 cm diam)
> PVC elbows for frames (40)
> Copper wire (12-gauge, insulated and uninsulated)
> Large cable ties (50)
> Either five 6-volt or 12-volt solar-powered fence chargers (Parker McCrory Manufacturing Company; http://www.Parmak.com). Voltage of fence charger will depend on initial field trials that assess response of macroconsumers to electric charge in your study stream (see above)
> Unglazed clay tiles (60 tiles, each 7.5×15 cm)
> Medium-sized binder clips
> Monofilament nylon line

Field and Laboratory Materials (see Chapter 17 for materials to conduct periphyton analyses)

>   70% ethanol
>   Forceps
>   Net or sieve ($\leq$250-μm mesh)
>   Paper labels
>   Squirt bottles
>   Whirl-pak® or Ziploc® sample bags

Field Equipment

>   Current velocity meter (optional)
>   Light meter or spherical densiometer (optional)

Laboratory Equipment

>   Desiccator
>   Dissecting microscope
>   Drying and ashing ovens
>   Electronic balance ($\pm$0.1 mg)
>   Filtration apparatus (vacuum pump, filter funnel, Erlenmeyer filter flask, tubing)
>   High-speed centrifuge
>   Spectrophotometer or fluorometer

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Predator-Prey Interactions

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I. INTRODUCTION

Streams may be viewed as open, nonequilibrium systems, having multiple patches connected by migration (Cooper *et al.* 1990, Palmer *et al.* 1996, Forrester *et al.* 1999; see also Chapter 21). Since most theory describing predator effects on prey communities has been developed for closed, equilibrium systems (e.g., Slobodkin 1961), historically, ecologists did not consider predation an important determinant of the structure of stream communities (Allan 1983a, 1983b, 1995). However, recent models (e.g., Caswell 1978, Nisbet *et al.* 1997, Diehl *et al.* 2000) predict that predation can have a major influence on prey populations in nonequilibrium systems, underscoring the value of studying predator-prey interactions in streams. As background for studies on predator-prey interactions in streams, below is an introduction to the types of effects that predators can have on prey populations and communities, and some of the mechanisms that may explain those effects.

In many streams fish are the top predators, feeding on invertebrates on the stream bottom or drifting in the water column (Hyatt 1979; see also Chapters 21, 22, and 26). Depending on the system, fish consume representatives of many orders of stream insects including mayflies (Ephemeroptera), dragonflies and damselflies (Odonata), stoneflies (Plecoptera), hellgrammites (Megaloptera), caddisflies (Trichoptera) and true flies (Diptera), and other macroinvertebrates such as amphipods (Peckarsky 1982, Allan 1995; see Chapters 20 and 25). Most studies of predation in streams have been conducted on drift-feeding fish (e.g., trout: Metz 1974, Allan 1981, Healey 1984) or benthic-feeding stoneflies (e.g., Malmqvist and Sjostrom 1980, Allan 1982a, Molles and Pietruszka 1983,

1987, Peckarsky 1985, Walde and Davies 1987). Thus, less is known about the effects of other predators in streams.

Predators can affect prey populations and communities by direct predator-induced mortality or by direct and indirect effects on prey behaviors and life histories (Sih 1987, Strauss 1991). For example, predators can have indirect community level effects ("top-down" cascading trophic effects) if reducing prey abundance increases resources used by prey (Carpenter *et al.* 1987, Power 1990). Alternatively, predators can have direct but nonlethal effects on prey populations through predator-induced changes in prey behavior or life history (Peckarsky *et al.* 1993, 2002). In this case interactions between predators and prey that do not result in prey death can have negative consequences on prey population growth (McPeek and Peckarsky 1998). This may occur if predator-avoidance behavior is costly to prey in terms of lost feeding time, shifting to unfavorable food patches, or shifting to less favorable feeding times (Peckarsky 1996). Alternatively, prey may alter their development to reduce exposure to dangerous predators (Crowl and Covich 1990, Peckarsky *et al.* 2001). Thus, the impacts of predators in streams can be studied from two general perspectives: (1) effects of predator-induced mortality on prey populations and communities and (2) consequences of antipredatory behavior and life histories on prey fitness and prey population growth.

The effects of predators often depend on whether predators are selective (i.e., consume certain prey types disproportionate to their abundance). Community ecologists are interested in whether selective predation alters the relative abundance of prey, which often has indirect effects on other components of communities (Paine 1966, Connell 1975). Selective predation may result from concentration of predator search in the preferred habitat of the prey, selection of prey types most frequently encountered, active rejection of some encountered prey individuals, or differential prey vulnerability (Greene 1985, Sih 1987, Allan and Flecker 1988, Fuller and Rand 1990). These alternative mechanisms of selective predation can be differentiated by measuring predator-prey encounter rates, attacks per encounter, and captures per attack, which are the major components of the predator-prey interaction (Peckarsky *et al.* 1994).

Behavioral ecologists often focus on the significance of differential prey defenses (Cooper 1985, Greene 1985, Peckarsky and Penton 1989), while population and evolutionary ecologists study the fitness consequences of predator-induced changes in prey behavior or life history (Crowl and Covich 1990, Peckarsky *et al.* 1993). If strategies to avoid predation result in reduced prey fecundity, demographic models predict that predators may affect prey population growth more strongly via nonlethal effects than by predation (McPeek and Peckarsky 1998). These predictions have been corroborated by field studies (Peckarsky *et al.* 2001), and experiments (Peckarsky and McIntosh 1998, Peckarsky *et al.* 2002), suggesting that in streams, predator effects on prey behavior and life history may be more important than on prey mortality.

The impact of predators on different prey species depends on relative prey vulnerability, immigration rates, and tendency to emigrate from patches where predators are foraging (Peckarsky 1985, Lancaster *et al.* 1991, Forrester 1994). Thus, it is important to know the prey *exchange rate*, the rate at which prey move in and out of areas where predators are feeding (Cooper *et al.* 1990). In streams with fast flowing riffles and high invertebrate drift rates (see Chapter 21) predation may be swamped by prey immigration (Cooper *et al.* 1990, Englund 1997). In streams with low rates of prey immigration, or where predators induce high rates of prey emigration (Sih and Wooster 1994), predators may have more substantial impacts (Cooper *et al.* 1990). In one striking example, a large-scale, long-term reduction of natural trout densities in a high-altitude stream had no detectable effects

on the abundance of invertebrate prey, possibly because high prey mobility obscured the effects of consumption by predators (Allan 1982b). Thus, the influence of predation on organisms living in open systems with extensive dispersal needs to be assessed relative to other influences on population dynamics (Palmer *et al.* 1996).

The purpose of this chapter is to introduce students and researchers to the study of predator-prey interactions from community, behavioral, and population perspectives. Methods are presented to (1) compare field measurements of predator consumption (gut contents) to estimates of prey availability to generate hypotheses on selective predation at the community level (Basic Method 1); (2) test those hypotheses by conducting mechanistic predation experiments to determine whether predators feed selectively on certain prey species (Basic Method 2); (3) conduct behavioral experiments to distinguish which components of predator-prey interactions explain observed patterns of selective predation (Basic Method 3); (4) compare field estimates of prey mortality to experimentally derived predation rates to generate hypotheses regarding the potential for predation to explain patterns of prey abundance in nature (Advanced Methods 1 and 2); and (5) measure effects of predators on prey behavior and life histories (Advanced Method 3).

II. GENERAL DESIGN

A. Site and Species Selection

The feasibility and specifics of these methods will depend on access to low (first–third) order rocky-bottom streams with riffle habitats containing abundant populations of large predatory stoneflies (Plecoptera: families Perlidae or Perlodidae) and potential mayfly prey species (Ephemeroptera: families Baetidae, Leptophlebiidae, Heptageniidae, Ephemerellidae). While it is possible to substitute other predatory taxa [e.g., benthic fish (see Kotila 1987), dragonflies, or hellgrammites] these methods were designed specifically for stonefly-mayfly interactions and, thus, have the highest probability of succeeding if those taxa are used. Basic Method 1 and Advanced Method 1 involve field collection of predators and prey, and will work best if predators are abundant (several predators per sample). For experiments (Basic Methods 2 and 3, and Advanced Methods 2 and 3) researchers should use the most abundant predator species and, for Basic Methods 2 and 3, three abundant alternative prey species — one overrepresented, one underrepresented, and one eaten in proportion to its availability in the predator's habitat (as determined by Basic Method 1). The prey species most abundant in predator diet should be used for Advanced Methods 2 and 3.

Predation experiments in Basic Method 2 and Advanced Method 2 can be carried out in enclosures placed in very shallow (<10 cm), moderately flowing (15–20 cm/s) riffles in the field, if such habitats are available and will not be disturbed overnight. Likewise, behavioral experiments (Basic Method 3 and Advanced Method 3) can be done in enclosures *in situ* but with less concern for disturbance, since they will not be left unattended. Alternatively, Basic Methods 2 and 3, and Advanced Methods 2 and 3 can be carried out in the laboratory if the researchers have access to dechlorinated water (e.g., well water or stream water) that can be distributed to replicate enclosures. However, best results will be obtained using circular, flowthrough enclosures set up streamside and using natural stream water.

B. Field-derived Electivity Indices — Generating Hypotheses for Community Level Effects

A simple method of estimating selective predation in the field involves comparing the proportion of prey in predator guts to relative prey abundance in the habitat (Chesson 1978). Although gut content data may provide an accurate record of undigested prey parts, there are many potential limitations to this method (see also Chapter 27). Variation in gut clearance time of different prey species (Hildrew and Townsend 1982) may lead to overestimation of prey with heavily sclerotized parts compared to soft-bodied prey. Partial consumption of prey may leave heavily sclerotized parts uneaten (Martin and Mackay 1982, Peckarsky and Penton 1985). Furthermore, ingestion of prey fragments, prey maceration, regurgitation during preservation, or alteration of gut contents by preservatives may also constrain our ability to quantify predator diets accurately from gut contents. Thus, gut contents show only part of what has been eaten, and could result in misinterpretation of the relative consumption rates of different prey species.

Field estimates of prey preferences also depend on the accuracy of estimates of prey abundance. A large literature deals with potential problems with the accuracy of benthic samples (Resh 1979 and see Chapter 20). Using samples of prey abundance to estimate their availability to predators assumes that (1) samples accurately reflect relative prey densities; (2) predators encounter prey at rates commensurate with measured prey density; and (3) the predator perception of available prey is the same as that of the investigator. Little is known about natural predator-prey encounter rates (Peckarsky *et al.* 1994) or predator perception of available prey in streams (O'Brien and Showalter 1993), since it is difficult to observe stream predators in their natural habitat. Consequently, hypotheses of differential predation based on data obtained by this field approach should be tested using other methods (see following).

To estimate selectivity from field data, investigators compare the relative importance of each prey item in predator gut contents to its relative abundance in the habitat. The simplest approach (correlation) involves comparing the ranks of prey types in the predator guts and in the habitat using Spearman's rank correlation analysis (Siegel 1956). A significant positive correlation indicates no selectivity (similar ranks of prey items in the diet and in the environment); no correlation or significant negative correlations suggest selective predation (feeding is weakly or strongly disproportionate to availability of prey in the environment). A second approach involves calculations of electivity indices (Ivlev 1961, Jacobs 1974, Chesson 1978, see also situation-specific modifications in Johnson 1980, Lechowicz 1982), which compare the proportion of each prey item in the predator's gut (r_i) to its proportion in the habitat (p_i). For preferred prey, $r_i > p_i$; $r_i < p_i$ suggests avoidance or prey unavailability; and if $r_i \sim p_i$, that prey item is being consumed in proportion its abundance in the environment. This method generally provides no significance tests (but see Lechowicz 1982) but can be used to compare the strengths of selection or avoidance among alternative prey. Finally, remember that this approach can only be used to hypothesize positive or negative selection for certain prey species, and that further tests are necessary to determine the reasons why specific patterns were observed.

C. Predation Experiments — Testing Hypotheses for Community Level Effects

An effective way to test hypotheses on selective predation generated from field data is to conduct predation experiments in the field or the laboratory, providing data that reveal cause and effect. Known numbers of alternative prey with contrasting patterns

of selectivity suggested by field data can be offered to predators in replicate enclosures closed to migration. Short-term prey disappearance rates can be measured and compared to prey disappearance from control enclosures containing the same prey numbers but no predators. Prey mortality rates (Dodson 1975) can be calculated for each prey species, and significance tests (analysis of variance) can be used compare predation rates among prey species (Peckarsky and Penton 1989). However, researchers must be aware of potential artifacts of enclosures (Hulberg and Oliver 1980, Peckarsky and Penton 1990), and interpret experimental data accordingly. For example, *in situ* mesh cages can slow stream flow and cause deposition of fine sediments, altering the behavior of predators or prey (Peckarsky 1985, Walde 1986). Nonetheless, correspondence between field and experimental data provide a powerful tool for answering questions about selective predation. If data from the two methods disagree, the investigator is then challenged to identify the artifacts biasing one or both methods (Peckarsky *et al.* 1997).

D. Behavioral Experiments — Testing Mechanisms for Behavioral Effects

Prey that are positively selected, avoided, or eaten in proportion to their abundance can be observed in enclosures to determine the precise components of the predator-prey interaction that cause the observed patterns. The biggest challenge in this approach is to design an enclosure similar to the natural environment that enables researchers to view interactions (e.g., Peckarsky *et al.* 1994). If compromises are made to observe organisms that are nocturnal or hidden under rocks, data need to be interpreted with caution. Removal of stream organisms from natural conditions and the presence of an observer can affect their behavior (Peckarsky 1983, Wiley and Kohler 1984). With this in mind, observers can conduct timed, replicated trials with one predator and identical densities of alternative prey species recording the numbers of predator-prey encounters, attacks, and captures per trial. Comparisons among prey species using significance tests (analysis of variance) enable researchers to determine whether prey taxa are selected or avoided on the basis of differences in encounter rates, attacks per encounter, or captures per attack. These data indicate whether prey selection is due to active choice by the predator or a passive consequence of prey attributes or behavior (Peckarsky and Penton 1989).

E. Field Estimates of Prey Mortality Rates — Generating Hypotheses for Population Level Effects

Methods developed by Kerans *et al.* (1995) can be used to estimate per capita daily mortality from sequential samples of one or more prey species in one or more stream sites. For this method sites should be sampled at one-week or two-week intervals during time periods when density of one cohort of an abundant prey species steadily declines, but before adult emergence could account for prey losses. Prey larvae can also be classified by developmental stage to estimate the development time of a cohort in each stream, which will also enable researchers to estimate the probability of surviving the larval stage.

F. Experimental Estimates of Predator-induced Mayfly Mortality — Testing Hypotheses for Population Level Effects

Investigators can estimate the proportion of larval mortality at each site that could be attributed to predation using predation experiments (similar to C above) and field

densities of predators (sampled at the same time as prey densities — E above) to estimate potential prey mortality that could be attributed to predation. Species pairs used in predation experiments should reflect known predator-prey interactions (B above), and temporal and spatial overlap between predators and prey species (Peckarsky and Cowan 2002). Functional response experiments (Kerans *et al.* 1995, Elliott 2003) measure the number of prey eaten across several prey densities to calculate daily predator-induced per-capita prey mortality rates, which can be compared to natural mortality rates estimated with field data (E above).

G. Effects of Predators on Prey Behavior and Life History — Testing Hypotheses for Non-lethal Fitness Effects

Experimental protocols similar to section D can be used to test the effects of non-feeding predators on prey behavior or life history by introducing cues from foraging predators into arenas without allowing predators to consume prey. Mouthparts of stoneflies can be glued with Barge Cement, if they forage naturally in chambers with mayflies (Peckarsky *et al.* 1993). Chemical cues from brook trout feeding in separate chambers can be introduced into chambers containing mayflies (McIntosh and Peckarsky 1996). Using these protocols, feeding behavior (foraging on rock surfaces, drift among different rocks) and life history parameters (growth rates, development times, size at maturity) can be compared statistically with and without predator cues.

III. SPECIFIC METHODS

A. Basic Method 1: Electivity Indices

1. Field Protocols

Researchers should collect invertebrates using a sampler designed for sampling in stream riffles (D-frame net, Surber sampler, Hess sampler; see Chapter 20). Methods can be standardized either by sampling the same microhabitat or by using the same effort for each sample (or both). If available to the researcher, an electrofishing machine may be equipped with a smaller anode and placed inside a Hess Sampler to take samples of benthic invertebrates (Taylor *et al.* 2001).

1. Using methods described in Chapter 20 or in Taylor *et al.* (2001) collect macroinvertebrates from a prescribed area of substrate (including large cobbles) in a shallow (<30 cm) riffle with moderate flow (20–30 cms^{-1}). The size of the area disturbed, and the number of samples taken depends on the productivity of the stream with a goal of collecting at least 100 individuals. Samples may be combined for analysis or kept separate to preserve replication and estimate variation.
2. Place each sample in a shallow pan, and use forceps to remove and preserve all large predatory stoneflies in a jar or whirlpack containing 70% ethanol. If no predatory stoneflies are collected, discard the sample (no useful information will be obtained). Preserve the rest of the sample after removing large bits of detritus and inorganic sediment. One of the major advantages of the "electro-bugging" method is that samples contain much less debris, and can be sorted more efficiently than standard "kick" samples (Taylor *et al.* 2001).

2. Laboratory Sorting, Counting, and Reference Protocols

1. Sort each sample and record the numbers of individuals collected of each predatory stonefly and prey taxon on Table 24.1. Since stoneflies primarily eat midges, black flies, and mayflies (Peckarsky 1985), or sometimes caseless caddisflies (Stewart and Stark 2002), other taxa need only be identified to order (see Chapter 20). However, blackflies (Simuliidae), midges (Chironomidae), and mayflies should be identified at least to family (especially Baetidae, Leptophlebiidae, Heptageniidae, and Ephemerellidae).
2. Prepare a reference collection of the invertebrates found at the stream to facilitate this process and minimize errors in identification.
3. Calculate the total numbers of each prey taxon and the proportion of the total individuals in all samples combined (p_i), and record data on Table 24.1. Alternatively, proportions of prey taxa may be calculated for each sample to estimate variability of relative prey abundance.

3. Protocol for Gut Content Analyses

1. Use two pairs of forceps to pull the head from the prothorax of each individual of the most abundant predatory stonefly taxon. The foregut, which should remain intact and attached to the head, can then be dissected and examined for recognizable prey parts. If the foregut does not remain attached to the head, dissect the thorax (through the ventrum) and anterior abdomen to extract the foregut. Since large predatory stoneflies swallow their prey whole, prey should be identifiable, provided a short time has elapsed since the predator's last meal.[1]
2. Use the reference collection of potential prey taxa or taxonomic references to identify prey in the predator's foregut. Prey fragments (claws, mandibles, head capsules, etc.) can be identified by comparison to whole specimens.
3. Record numbers of each prey taxon found in each predator gut on Table 24.1; calculate totals for each taxon, and the proportion of the total prey individuals for all predators combined (r_i). Alternatively, stoneflies may be analyzed separately to estimate variation in predator diets.

4. Data Analysis

1. Using the combined samples (Table 24.1) compare the fractional composition of each item (i) in the guts of the stoneflies (r_i) to its fractional composition in the available food supply (p_i) using Ivlev's Electivity Index (1961):

$$E_i = (r_i - p_i)/(r_i + p_i) \qquad (24.1)$$

Values of E_i can range from −1 to +1 indicating avoidance to preference, with values near zero indicating that the prey item is eaten in a similar proportion that it was collected in the environment. Record the electivities for each prey taxon on Table 24.1.

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[1] For best results, samples should be taken in the morning because most predatory stoneflies are nocturnal feeders (Peckarsky 1982), and food items in the gut will be less digested.

TABLE 24.1 (Basic Method 1) Benthic Data and Predator Guts to Calculate Spearman Rank Correlation Coefficients or Ivlev's Electivity Index for Selective Predation. Gray cells are not applicable.

| Samples | Number in benthic samples | | | | | | Rank: | Number in predator guts | | | | | Rank: | (Eq. 24.1) |
	1	2	3	...n	total	p_i	Habitat	1	2	3	...n	total	r_i	Diet	Ivlev's E_i
Predator taxon:															
Prey taxa:															
Baetidae															
Ephemerellidae															
Heptageniidae															
Leptophlebiidae															
Other Ephemeroptera															
Plecoptera															
Trichoptera															
Chironomidae															
Simuliidae															
Other Diptera															
Total Prey															

2. Use these combined data to prepare a bar graph illustrating the electivities of each taxon, placing prey taxa on the horizontal axis in order of decreasing electivity. Alternatively, electivities may be calculated for predators in each benthic sample separately, in which case mean and variation around the mean can be plotted for each prey taxon.

3. Also using data from the combined samples, calculate a Spearman Rank Correlation Coefficient (Siegel 1956) to test for significant correlation ($p < .05$) between the ranks of potential prey taxa in the diets and in the habitat of the stoneflies (see Table 24.1).

B. Basic Method 2: Predation Experiments to Test for Selective Predation

1. Protocols for Field or Laboratory Trials

1. Collect predators and prey in the field and hold predators in aerated, cooled (10–15°C) or flowing water without prey for at least 24 hr to standardize hunger levels. For best results, minimize handling; predators should be handled with soft forceps, and prey individuals can be transferred between containers using large mouthed plastic pipettes.

2. Each set of replicates should include six enclosures (single prey trials), two each per three prey species containing 15–20 prey and either one predatory stonefly (predator treatment) or no stonefly (control).[2]

3. Choose three prey species from the available taxa identified in Basic Method 1. Preferably, they should include one overrepresented (positive electivity), one underrepresented in stonefly diets (negative electivity), and one eaten in proportion to its availability (electivity ∼ zero). If three prey species are not available, this method can be accomplished with two prey species. Mayfly species are preferable, because they are easier to handle and manipulate than dipterans or caddisflies, which tend to slip through meshes (midges) or spin silken threads in which stoneflies get tangled (black flies and caddisflies).

4. Field enclosures should be rectangular with upstream and downstream ends covered with mesh (∼800-μm openings: small enough to retain prey but large enough to minimize clogging). A simple design is a fabricated plexiglass box (Figure 24.1), but cheaper materials may be used, such as Rubbermaid® shoe boxes, with openings cut in the sides and screened with Nitex® attached to walls with hot-melt glue.

5. The floor of each enclosure should be covered with a standardized number of cobbles ranging from 5–15 cm in diameter with the same size distribution in each enclosure. It is best to use natural algal-covered stream substrata from which all invertebrates have been carefully removed. Such cobbles also provide food for prey, refuges for predators and prey, and anchor enclosures to the streambed.

6. The best design for laboratory enclosures is circular (10–15 cm diameter), which reduces edge effects. These can be made of plexiglass (e.g., Figure 24.2) or modified cylindrical food containers, and powered by water (Walde and Davies 1984, Peckarsky and Cowan 1991) or air pressure (Wiley and Kohler 1980, Mackay 1981). Air pressure necessitates recirculation of water and some type of refrigeration; water-powered chambers can use cold running water and central mesh-covered

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[2] Number of replicates of treatments and controls should be maximized but may depend on feasibility. Number of prey included in each chamber will depend on the size of the chamber and should fall within the range of observed densities for each prey taxon in the field.
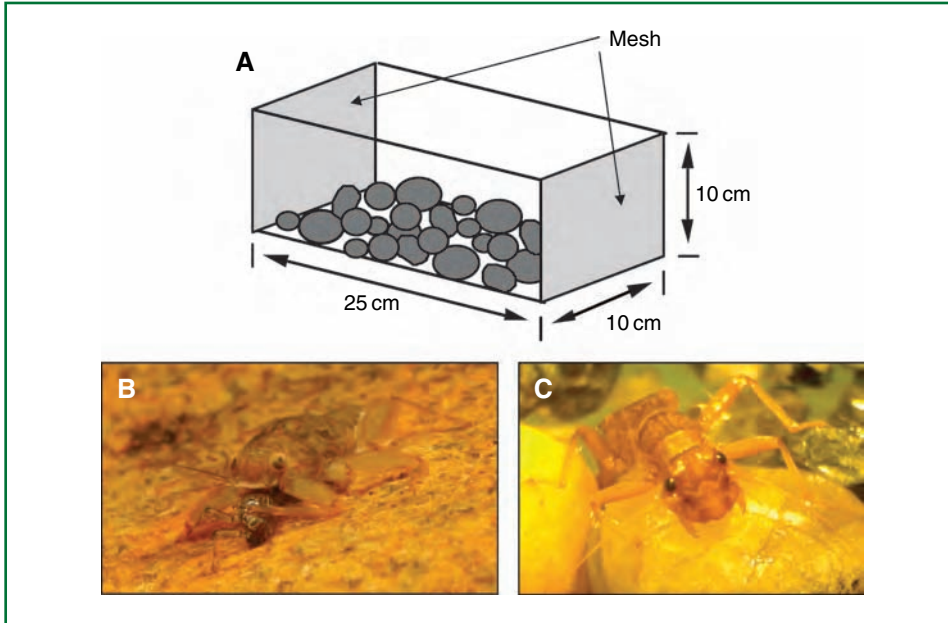
FIGURE 24.1 *In situ* enclosures. (A) Drawing of rectangular chambers for *Basic Method 2* (predation choice trials) that can be used in the field (from Peckarsky and Penton 1989). Shaded areas represent screen mesh or Nitex®. Photographs of (B) *Drunella doddsi* consuming *Baetis bicaudatus* (photo: Angus McIntosh), and (C) *Megarcys signata* foraging (photo: Michael Benton).
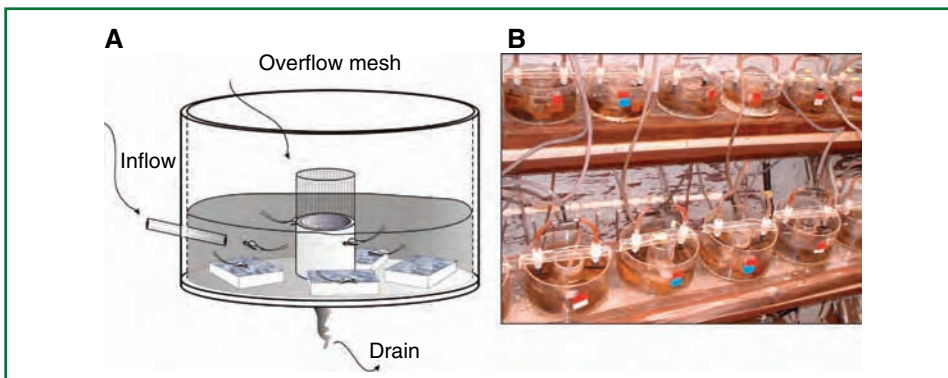
FIGURE 24.2 Small flowthrough streams for predation experiments. (A) Drawing (by Peter Ode) and (B) photograph of two different designs of circular chambers for *Basic Method 2* and *Advanced Methods 1 and 2* (predation experiments) that could be used in the laboratory or streamside.

standpipes to regulate water levels. These designs can be modified depending on facilities available, but cool temperatures (10–15°C) and good oxygenation are essential conditions to facilitate stonefly foraging. Again, natural algal-covered substrata can be collected from the stream and used for food and refuges for prey and predators.

7. Allow predators to feed in enclosures overnight or for 24 hr. It is advisable to conduct a pilot trial to determine the time during which predators eat detectable numbers of prey but do not deplete prey in any chambers (about 10–50% prey consumption is optimal). After the trial, record the numbers of prey remaining in each chamber on Table 24.2.

2. Data Analysis

1. Calculate a mean correction factor for losses of each prey species from controls, which are due to factors other than predation (see Table 24.2). Subtract that correction factor from numbers of prey missing from treatments with predators.
2. Calculate instantaneous prey mortality rates (m) for each prey species tested using the equation:

$$m = [\ln N_o - \ln N_f]/t \qquad (24.2)$$

   where N_f = final density of prey remaining in chambers (corrected for average number lost from all controls with that species), N_o = initial prey density (e.g., 15–20 individuals), and t = duration (days) of the trial (Dodson 1975). The units of this parameter (m) are prey mortality per prey per predator per day, which takes into account exploitation of prey over the time of the trial. Record these values on Table 24.2.
3. Using the data recorded on Table 24.2, prepare a bar graph of the mortality rate m, showing mean ± SE for each of the three prey species. Use a one-way analysis of variance (ANOVA) and multiple comparisons tests (e.g., Sokol and Rohlf 1995) to test for significant differences in predation rates among the three prey species. Compare these results to those predicted by hypotheses generated from the field data (Basic Method 1).
4. Alternatively, plot mean ± SE mortality rates in controls and predator treatments, and use a two-way ANOVA to compare mortality rates of prey species in controls versus predator treatments to test for significant predator-induced mortality on each species.

C. Basic Method 3: Behavioral Experiments to Test Mechanisms of Selective Predation

1. Field or Laboratory Trials

Using the same combinations of predators and prey as in Basic Method 2, conduct behavioral trials to determine which components of the predator-prey interaction are responsible for observed patterns of selective predation.

1. Containers used in Basic Method 2 can be used for these trials, except that substrate will have to be modified for viewing of behavior. Circular plexiglass chambers with natural substrata can be placed in elevated plexiglass trays and viewed by observers from the top and bottom (Figure 24.3). If such chambers are not available, use of

TABLE 24.2 Data (Basic Method 2) for Calculating Predator-Induced Prey Mortality (m).

	No. missing from controls			No. missing from predator treatments			Mortality due to predation		
	Prey sp. 1	Prey sp. 2	Prey sp. 3	Prey sp. 1	Prey sp. 2	Prey sp. 3	Prey sp. 1	Prey sp. 2	Prey sp. 3
Replicate no.									
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
...n									
	Correction factors			Corrected number missing			Calculate using Eq. 24.2		
Mean									
SE									
N									
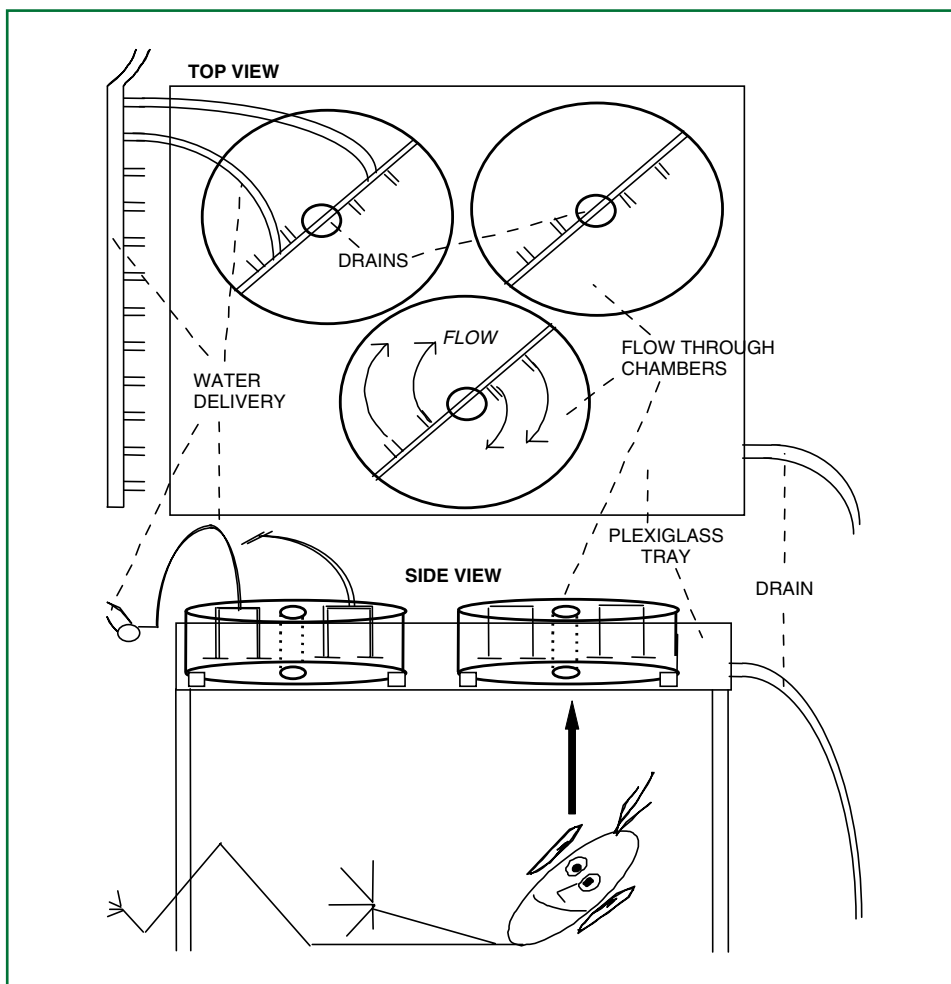
FIGURE 24.3 Drawing of plexiglass arenas for *Basic Method 3* (behavioral observations of predator-prey interactions) that could be used in the laboratory or field (from Peckarsky *et al.* 1994).

gravel or sand into which prey and predators cannot burrow is an alternative, but data may be biased by unnatural conditions (no refuges for predators or prey).

2. Each replicate should consist of three 10-min trials observing one 24-hr starved predator with 15–20 (same density as in Basic Method 2) individuals of each prey species one at a time (i.e., single prey species trials). Order of prey species observed should be randomized and replication should be maximized. Mixed prey species combinations can be used here and in Basic Method 2, but statistical analyses become complicated, necessitating the use of MANOVA (Peckarsky and Penton 1989). Trials should be conducted during natural feeding times of predators. If this is at night (typical for predatory stoneflies), observers should observe interactions using a flashlight covered with red acetate, and determine beforehand whether red light affects the behavior of stonefly or mayfly species (Peckarsky and Cowan 1995,

Peckarsky 1996). If replicates need to be run on different days, a repeated measures ANOVA should be used to test for effects of day on predation rates.

3. Observe each predator and set of prey only once, using careful handling techniques outlined in Basic Method 2. For each trial, record the number of encounters, attacks per encounter, and captures per attack on Table 24.3. If there are no encounters, a new predator should be observed, because there will be no useful data obtained from an inactive predator. However, the trial is useful if there are encounters but no attacks, but captures per attack are undefined.

2. Data Analysis

1. Prepare three bar graphs, one each for encounters, attacks per encounter, and captures per attack, to illustrate and compare the mean ± SE values (Table 24.3) for each of the three species.
2. Using the data from all observations (Table 24.3), compare each of the three parameters (i.e., encounters, attacks per encounter, and captures per attack) among the three different prey species using a one-way ANOVA and multiple comparisons tests.

D. Advanced Method 1 — Field Estimates of Prey Mortality Rates

Sequential samples of single cohorts of a prey species can be used to estimate loss rates over time under natural stream conditions. Observed loss rates may be attributed to mortality only if immigration and emigration are similar. Thus, investigators should also estimate drift into and out of a selected study reach to test this assumption (Chapter 21).

1. Field Collections

1. Select a time when 3–6 weekly or biweekly samples can be taken during the period of growth and development for one cohort of the most abundant prey species (Table 24.1).
2. On each day take 3–6 quantitative benthic samples using a fine mesh (200 μm) net and one of the devices described in Basic Method 1. Preserve all invertebrates in 70% ETOH. Maximize replication in time and space.

2. Laboratory Processing of Invertebrates

1. Record the two-dimensional surface area of the sampler so that predator and prey densities can be estimated (see Table 24.4A).
2. Record the number of predatory stoneflies (same species as used for Basic Methods 1–3) on Table 24.4A. Numbers of predators collected in all benthic samples may be combined for each date, or average number of predators per sample may be recorded. If samples are combined, record the total area sampled (area of one sampler × number of samples per site per date).
3. Count and stage mayfly prey using wing pad development (Stage I = no wing pads, Stage II = wing pads wider than long, Stage III = wing pads longer than wide, and Stage IV = black wing pads; Peckarsky *et al.* 2001). Calculate prey density per

TABLE 24.3 Data (Basic Method 3) on Number of Encounters, Attacks per Encounter, and Captures per Attack.

	Number of encounters			Attacks per encounter			Captures per attack		
	Prey sp. 1	Prey sp. 2	Prey sp. 3	Prey sp. 1	Prey sp. 2	Prey sp. 3	Prey sp. 1	Prey sp. 2	Prey sp. 3
Replicate no.									
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
...n									
Mean									
SE									
N									

TABLE 24.4 **Data (Advanced Methods 1 and 2) to Compare Natural Prey Mortality to Predation Rates.**

A. Advanced Method 1
Prey taxon:

Date	Cum.[1] Days	Area sampled (m^2)	No. predators	Predator density (N_P)	No. prey collected in benthic samples					Prey Density
					Stage I	Stage II	Stage III	Stage IV	Total	
1	0									
2										
3										
4										
5										
6										
…n										

[1]Total days from date 1 – n

mean	
SE	
N	

[2]Assumes density declines from date 1 to n.

Daily Prey Mortality[2] (Eq. 24.2 – Field modification)

B. Advanced Method 2

Daily Per Capita Prey Mortality (Field)	Prey Density[3]	Predator-induced prey mortality[4] (M_P from experiments – Eq. 24.2)	Predation Rate (Experiments) Adjusted by Predator Density (Field)	Ratio of Adjusted Predation Rate to Field Mortality
	5			
From Advanced Method 1	10			
(Table 24.4A)	15			
	20			
Averaged over all prey densities:				

[3]Adjust accordingly if different prey densities are used.　　　　[4]Calculated as in Table 24.2

sample or combine all samples using the appropriate area sampled as described for predators above (see Table 24.4A).

3. Data Analysis

1. Estimate daily per-capita mortality (m) (as in Kerans *et al.* 1995):
   $m = (\ln [N]_t - \ln [N]_{t+1})/d$ (modification of Eq. 24.2), where N = density of all stages combined; t and t+1 = the first and last dates of time series of samples during which density steadily declined, but before adult emergence could account for losses; and d = days between samples. Record that value in Table 24.4A.
2. Depending on the stage structure of the mayflies during the sampling period, and the synchrony of development, investigators may also be able to estimate the development time (D) of larvae as the number of days to advance from stage II–stage IV. If this is possible, the probabilities of surviving the larval stage (K) can also be estimated.

$$(K = e^{-mD}) \tag{24.3}$$

assuming constant mortality rate (m) during a larval period of duration D.
3. Estimates of mortality, development time, and probability of surviving the larval stage can be plotted using bar graphs of means and standard errors to illustrate comparisons of taxa or streams. These parameters can be compared among species of mayflies or populations in different types of streams (e.g., fish and fishless) using MANOVA on log-transformed data.

E. Advanced Method 2 — Experiments to Test for Predator-induced Mortality Rates at Different Prey Densities

Natural larval mortality (measured in Advanced Method 1) of different prey species or sites can be compared to estimates of predation rates using instantaneous attack rate coefficients from small-scale functional response experiments (Kerans *et al.* 1995) combined with field estimates of natural predator densities (from Advanced Method 1). Investigators could also use trout as predators in larger arenas (Figure 24.4) and estimate trout densities by electrofishing (Chapter 22). The following protocol describes methods for predatory stoneflies and mayfly prey.

1. Design of Predation Rate Experiments (Functional Response)

1. Conduct overnight or 24-hr predation trials using the same protocol as in Basic Method 2 (preferably the circular chambers — Figure 24.2, which provide more accurate estimates of mortality due to predation), but this time varying the prey density (e.g., 5, 10, 15, and 20 prey per chamber) with one stonefly predator, and the same prey densities with no predators as controls.
2. Species pairs and densities used in experiments should reflect known prey preferences (Basic Method 1), known temporal and spatial overlap between predators and prey species (e.g., Peckarsky and Cowan 1995), and the natural range of prey densities (Advanced Method 1).
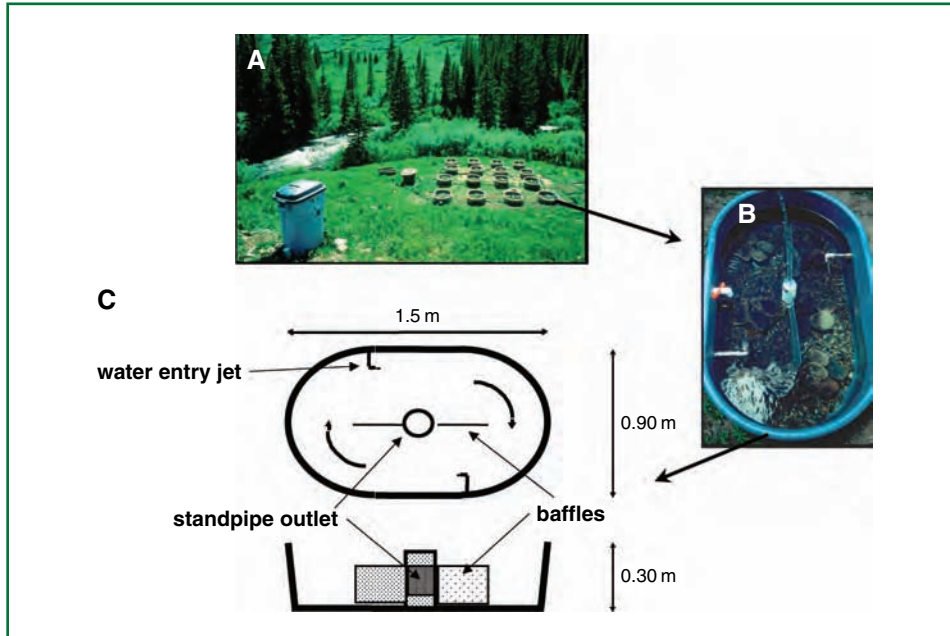
FIGURE 24.4 Large artificial streams useful for *Advanced Methods 2 and 3* (predation experiments with fish). (A) Artificial streams at Rocky Mountain Biological Laboratory, Colorado, are gravity-fed with water from a nearby fishless stream, which provides a source of fishless water for controls. Fishless water can also be gravity-fed to a 110-L holding tank (shown at left) containing two brook trout and then dripped into tanks allocated to a fish-cue treatment. Alternatively, fish can be added directly to streams. (B) Single artificial stream; fish water drips in through spout with orange ribbon. (C) Schematic diagram of an artificial stream in top and side views (from McIntosh and Peckarsky 1996).

2. Data Analysis

1. As in Basic Method 2 (Table 24.2), calculate the daily predator-induced per-capita prey mortality rates (M_p) using a modification of equation 24.2:
$M_P = (\ln [N_I] - \ln [N_F])(P)^{-1}(d^{-1})$, where N_I = initial prey density, N_F = final prey density, P = predator density, and d = days of the feeding trial. Use the area of the experimental unit to estimate predator and prey densities. Record estimated predation rates for each prey density treatment on Table 24.4B.
2. To compare these estimated predation rates to natural prey mortality estimated in a particular stream, first adjust predator-induced mortality (M_p from Table 24.4B) by average predator density measured in that stream (N_p from Table 24.2A, Advanced Method 1); then calculate the ratio of the adjusted predation rate to the loss rate of prey from that stream (m from Advanced Method 1) as $M_p \times N_p/m$. Record this value in Table 24.4B. If the predation rates at each prey density differ, select the prey density that best approximates that of the study stream. Otherwise, use the average estimated predation rate (Table 24.4B).
3. To compare multiple streams or multiple prey species or predator species, the ratios of adjusted predation rates to total prey mortality can be compared graphically and statistically using ANOVA on transformed data or nonparametric analysis of variance.

F. Advanced Method 3 — Experiments to Test Predator Effects on Prey Behavior and Life History

1. Design of Experiments

1. Set up replicate circular arenas similar to those used in Basic Methods 2 and 3 (Figure 24.2) using dechlorinated water (well water or stream water) in the laboratory or preferably by diverting natural stream water into streamside artificial streams (Peckarsky and Cowan 1991), which enables natural light and temperature regimes to be maintained.
2. Add 5–10 prey of a selected species to chambers with algal-covered natural rocks, or unglazed tiles can be substituted for ease of viewing. For behavioral trials, arenas should be left uncovered. If rearing prey to maturity, arenas should be covered with mesh emergence nets that allow light to penetrate.
3. To measure effects of stonefly predators on prey behavior/life history, use a thin wire or toothpick to place a small drop of Barge Cement on the mouthparts of a stonefly while it is anesthetized in a weak suspension of alka seltzer and water (Peckarsky *et al.* 1993). Allow glued stoneflies to recover in a holding chamber before using them in experiments. To start the experiment, place one stonefly in each chamber randomly allocated to the predator treatment, and a small piece of gravel with Barge Cement in chambers allocated to controls.
4. Observe and record feeding behavior (instantaneous scan of numbers of individuals foraging on the surface of substrates) or drift behavior (number drifting per unit time) of prey several times during a 24-hour period in chambers with and without glued stoneflies. Nighttime observations should be made using dim red light. Numbers of stoneflies visible foraging should also be recorded and compared to known natural feeding periodicity of the predators (determined in preliminary observations with nonglued stoneflies.)
5. To measure effects of glued stoneflies on prey life histories, prey should be reared to maturity (black wing pad — Stage IV) under these same treatments, and then preserved for analysis of size and fecundity (numbers of eggs per female).
6. Using a similar experimental design, chemical cues from brook trout feeding in separate chambers can be dripped to experimental arenas to test the effects of those cues on prey behavior and life history. Small chambers (Figure 24.3) allow greater replication, but larger chambers (Figure 24.4) provide a more realistic environment in which to measure prey life histories and behavior (McIntosh and Peckarsky 1996, Peckarsky and McIntosh 1998).

2. Data Analysis

1. Numbers of prey individuals foraging on rock surfaces and prey drift rates can be compared between predator treatments and controls graphically and statistically using MANOVA on multiple, interdependent response variables, and subsequent ANOVAs on individual response variables if the MANOVA is significant (Peckarsky and McIntosh 1998). Data should be transformed to meet the assumptions of parametric statistical tests.
2. Similarly, life history parameters (i.e., growth rates, development times, and size at emergence) can be compared graphically and by MANOVA (see Peckarsky *et al.* 1993) to test whether prey life histories respond to predator cues.

IV. QUESTIONS

1. What are the strengths and limitations of field-generated electivity indices? Predation experiments? Behavioral observations?
2. What hypotheses were suggested by the electivity indices or by correlations between gut contents and benthic data? Did these methods generate the same hypotheses?
3. What can you conclude about selective predation by stoneflies from predation experiments?
4. What did behavioral experiments reveal about the importance of encounter rates, attacks per encounter, and captures per attack as mechanisms explaining patterns of selective predation by stoneflies?
5. Is prey selection by stoneflies active or passive? Explain.
6. Are data from different methods to test for selective predation consistent? Describe any discrepancies. If data are not consistent, what conclusions would you draw? Do you trust some methods more than others? Why?
7. Why should investigators include controls and replication when designing experiments?
8. Were predation rates (estimated by functional response experiments) high or low compared to prey mortality observed in the field? What are the implications of your findings for the potential of predators to regulate prey populations in nature?
9. What are the potential fitness costs of lower growth rates, longer development times, and/or smaller size at maturity associated with avoiding predators? Alternatively, how might prey increase their fitness by accelerating their development, even if they emerge at smaller sizes in streams with dangerous predators? (Consider probability of surviving the larval stage.)
10. What did behavioral observations tell you about the possible mechanisms of observed effects of predator cues on prey life history?

V. MATERIALS AND SUPPLIES

For field collections (Basic Method 1 and Advanced Method 1)

> Collecting jars or whirlpaks
> Collecting devices (D-nets, Surber sampler, Hess sampler, electrobugging machine)
> Shallow sorting pans
> Plastic eyedroppers and soft forceps

Additional supplies for experiments (Basic Methods 2 and 3, and Advanced Methods 2 and 3)

> Enclosures/rearing chambers/observation chambers
> Holding chambers (for predators)
> Flashlights with red acetate to produce dim red light for nighttime observations
> Various plumbing supplies and a first-name basis with the local hardware store
> Water or air source (for circulating flow in chambers if trials are done in the laboratory)

Laboratory Equipment

    Petri dishes for sorting samples
    Dissecting microscope
    Dissecting forceps
    Invertebrate identification guide (see Appendix 20.1)

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Trophic Relationships of Macroinvertebrates

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I. INTRODUCTION

A major observation resulting from studies of aquatic invertebrate feeding (e.g., Berrie 1976, Cummins and Klug 1979, Anderson and Cargill 1987, Palmer *et al.* 1993a, Wotton 1994) is that, based on food ingested, essentially all aquatic invertebrates are omnivorous. For example, aquatic insects that chew leaf litter in streams, termed *shredders*, ingest not only the leaf tissue and associated microbiota, (e.g., fungi, bacteria, protozoans, microarthropods), but also diatoms (e.g., Plague and Wallace 1998) and other algae that may be attached to the leaf surface, as well as very small macroinvertebrates (e.g., first-instar midge larvae). For this reason, the trophic level analysis pioneered by Lindeman (1942), and used extensively in investigations of trophic relationships in marine and terrestrial communities, does not lend itself well to simple trophic categorization of stream macroinvertebrates (e.g., Coffman *et al.* 1971).

An alternate classification technique involves the functional analysis of invertebrate feeding based on morpho-behavioral mechanisms of food acquisition (Table 25.1). This functional feeding group (FFG) approach, described 30 years ago (Cummins 1973), has been modified in some details since then (e.g., Cummins 1974, Cummins and Klug 1979, Wallace and Merritt 1980, Merritt *et al.* 1984, 1996, 1999, 2002, Cummins and Wilzbach 1985, Merritt and Cummins 1996), but the basis of FFG relationships remains quite simple. FFGs are based on a direct correspondence between the categories of nutritional resources present in the environment and the populations of freshwater invertebrates that are adapted to efficiently harvest a given food resource. As the relative availability of the

TABLE 25.1 **General Classification System for Aquatic Insect Trophic Relationships.**[1]

FUNCTIONAL GROUP (GENERAL CATEGORY BASED ON FEEDING MECHANISM)	SUBDIVISION OF FUNCTIONAL GROUP			GENERAL PARTICLE SIZE RANGE OF FOOD (IN MM)
	Dominant Food	Feeding Mechanism	Examples of Taxa	
Shredders	Living vascular hydrophyte plant tissue	Herbivores — chewers and miners of live macrophytes	Trichoptera: Phryganeidae, Leptoceridae	>1
	Decomposing vascular plant tissue and wood — coarse particulate organic matter (CPOM)	Detritivores — chewers, wood borers, and gougers	Plecoptera: Nemouridae, Peltoperlidae Diptera: Tipulidae, Trichoptera: Limnephilidae, Lepidostomatidae Amphipoda	>1
Collectors	Decomposing fine particulate organic matter (FPOM)			
Filtering Collectors		Detritivores — filterers or suspension feeders	Trichoptera: Hydropsychidae, Diptera: Simuliidae	<1
Gathering Collectors		Detritivores — gatherers or deposit (sediment) feeders (includes surface film feeders)	Ephemeroptera: Ephemeridae Diptera: Chironominae	<1
Scrapers	Periphyton — attached algae and associated material	Herbivores — grazing scrapers of mineral and organic surfaces	Trichoptera: Glossosomatidae Coleoptera: Psephenidae Ephemeroptera: Heptageniidae	<1
Piercers-Herbivores		Herbivores — suck contents of algal cells	Trichoptera: Hydroptilidae	<1
Predators	Living animal tissue	Carnivores — attack prey, pierce tissues and cells, and suck fluids	Hemiptera: Belostomatidae Naucoridae	>1
	Living animal tissue	Carnivores — ingest whole animals (or parts)	Odonata, Plecoptera: Perlidae Megaloptera: Corydalidae Sialidae	>1

[1] *Modified from Merritt and Cummins (1996).*

basic food resources changes through space or time, there is a concomitant change in the corresponding ratios of the functional groups of co-occurring freshwater invertebrates. Thus, a limited set of feeding adaptations found in freshwater invertebrates is linked with their basic food resource categories.

The basic food categories for invertebrates in stream ecosystems are (1) *CPOM*, coarse particulate organic matter (particles greater than 1 mm in size), including litter accumulations consisting of leaves, needles, bark, twigs, and other terrestrial plant parts, large woody debris (i.e., large branches and logs), and macrophytes including macroalgae and rooted and floating vascular plants (see Chapters 13 and 18); (2) *FPOM*, fine particulate organic matter (particles ranging from 0.5 um to 1.0 mm in size) generally composed of unattached living or detrital material including that created through physical and biological reduction of CPOM and associated microbiota (see Chapter 12); (3) *periphyton*, predominantly attached algae (especially diatoms) and associated material growing on rock, wood, and plant surfaces (see Chapters 16 and 17); and (4) *prey*, all invertebrates captured by predators, predominantly small species and early instars of large species (see Chapters 19 and 24).

These four nutritional resource categories related to food acquisition mechanisms were chosen on the basis of the size range of the material (coarse or fine) and the general location of the food, such as attached to surfaces (periphyton), suspended in the water column (seston), deposited in the sediments, found in litter accumulations, or dispersed in the form of live invertebrates. This categorization also reflects (1) biochemical differences in the nutritional resources, such as presence of living chlorophyll in periphyton or microorganisms on CPOM, and (2) the major source of the food, such that whether it was either *autochtonous* (produced within the aquatic system; see Chapter 28) or *allochthonous* (produced from the streamside or riparian area; see Chapters 30 and 31).

The general FFG classification system for aquatic invertebrate trophic relations, in which taxa are categorized according to the different morphological-behavioral adaptations used to harvest nutritional resources, is presented in Table 25.1. Some representative FFG taxa are shown in Figure 25.1. These feeding mechanisms determine which of the categories represent the primary food resource: (1) *shredders* feed on CPOM; (2) *collectors* feed on FPOM; (3) *scrapers* consume periphyton; and (4) *predators* ingest prey. The functional groups described in this classification are analogous to *guilds*, which are groups of organisms using a particular resource class (Root 1973, Georgian and Wallace 1983, Hawkins and MacMahon 1989); thus, function in FFGs is defined as use of similar resource classes.

Within each FFG, there are obligate and facultative members. These can be different species or different stages in the life cycle of a given species. For example, it is likely that most aquatic insects, including predators, are facultative gathering-collectors in their early instars (Petersen 1974). Thus, the most reliable linkage between a food resource category (CPOM, FPOM, periphyton, prey) and macroinvertebrates is with the obligate forms in later instars. The distinction between obligate and facultative status is best described by the efficiency with which a given invertebrate converts a food resource into growth (Cummins and Klug 1979). It is important to understand that the same morpho-behavioral mechanisms can result in the ingestion of a wide range of food items, the intake of which constitutes herbivory (i.e., living plants; Gregory 1983, Lamberti and Moore 1984, Webster and Benfield 1986), detritivory (i.e., dead organic matter; Anderson and Sedell 1979, Wallace and Merritt 1980, Short 1983, Webster and Benfield 1986, Cummins *et al.* 1989, Palmer *et al.* 1993b), or carnivory (i.e., live animal prey; Allan 1983, Peckarsky 1984).

FIGURE 25.1 Representative stream functional feeding group taxa. **Shredders** (A. Diptera: Tipulidae, B. Plecoptera: Pteronarcyidae, C. Trichoptera: Limnephilidae); **Filtering Collectors** (D. Diptera: Simuliidae, E. Trichoptera: Hydropsychidae, F. Trichoptera: Brachycentridae); **Gathering Collectors** (G. Diptera: Chironomidae, H. Ephemeroptera: Ephemerellidae, I. Ephemeroptera: Ephemeridae); **Scrapers** (J. Ephemeroptera: Heptageniidae, K. Trichoptera: Glossosomatidae, L. Coleoptera: Psephenidae); **Piercers-Herbivores** (M. Trichoptera: Hydroptilidae); **Predators** (N. Plecoptera: Perlidae, O. Megaloptera: Corydalidae, P. Odonata: Gomphidae).

Although intake of different food types can be expected to change from season to season, habitat to habitat, and with growth stage, limitations in food acquisition mechanisms have been shaped over evolutionary time and these are relatively more fixed. For example, a scraper such as the mayfly *Stenonema* (Heptageniidae), whose mouthparts are adapted for shearing off organisms attached to surfaces, may ingest a variety of organic substrates
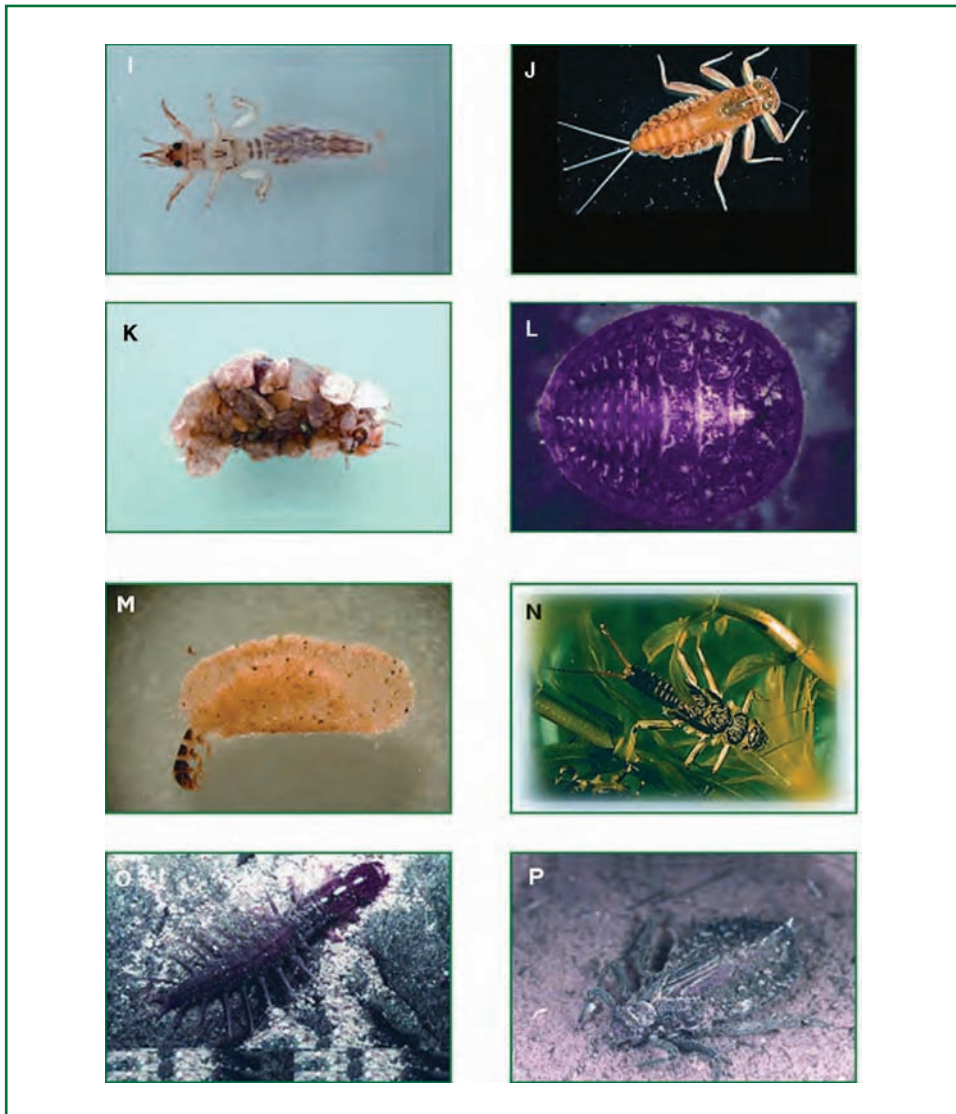
FIGURE 25.1 Continued.

within the restriction of its food-harvesting morphology without any change in behavior. As animals get larger, microhabitats scraped for food may change, or items scraped from surfaces may differ (Cummins 1980). Comparable (homologous) morphological structures that enable insects to scrape periphyton from substrates can be found in the very similar mandibles of taxonomically diverse groups such as the trichopteran families Glossosomatidae (saddle-case makers) and Helicopsychidae (snail-case makers), and the coleopteran family Psephenidae (water penny beetles). Such similarity of structure is a striking example of convergent evolution.

The FFG approach is informative in that it allows an assessment, numerically or by biomass, of the degree to which the invertebrate biota of a given stream is

dependent upon a particular food (nutritional) resource. It also makes apparent the linkages that exist between food resources and insect morphological-behavioral adaptations (Cummins 1974). As the relative dominance of various food resource categories changes, a corresponding shift in the ratios of the different functional feeding groups is expected. In this manner, invertebrate FFG analysis is sensitive to both the normal pattern of geomorphic and concomitant biological changes that occur along river systems from headwaters to lower reaches (e.g., Vannote *et al.* 1980), as well as to alterations

TABLE 25.2 Example of an Actual FFG Data Sheet for a Macroinvertebrate Sample from Rio dos Padres, State of Parana, Brazil.

Invertebrate Functional Group Assessment ~ Field Form

Stream: __Rio dos Padres__   Reach: _____ Drainage: _____ Date: __2/16/05__

Crew: __Cummins, Merritt, et al._____ H₂O Temp: __21°C__ @ Time: __9:10__ a.m.

Habitat Unit No.: ____1____ Habitat Type: __Cobble__

Sample Type (*circle one*): (Cobble) Wood Leaf Litter Fine Sediments Composite SAMPLE #: __1__-_____-____

Functional Group	Tally	Total	Identifiable Taxa
Shredders	ᚎᚎ ᚎᚎ ᚎᚎ ᚎᚎ ᚎᚎ III	23	Sericostomatidae = 1 *Gammarus* = 4 Gripopterygidae = 10 Calamoceratidae = 6 Leptoceridae = 2
Scrapers	ᚎᚎ ᚎᚎ ᚎᚎ ᚎᚎ ᚎᚎ II	27	Psephenidae = 1 Helicopsychidae = 1 Blephariceridae = 21 Gastropoda = 1 Elmidae (adults) = 3
Filtering Collectors	ᚎᚎ ᚎᚎ ᚎᚎ ᚎᚎ ᚎᚎ	25	Hydropsychidae = 24 Simuliidae = 1
Gathering Collectors	ᚎᚎ ᚎᚎ ᚎᚎ ᚎᚎ ᚎᚎ III	28	Baetidae = 10 Leptophlebiidae = 6 Elmidae (larvae) = 1 Chironomidae (not Tanypodinae) = 1 Leptohyphidae = 10
Predators	ᚎᚎ II	7	Dytiscidae = 1 Gerridae = 1 Limnocorinae = 2 Corydalidae = 2 Anisoptera = 1

Sample Type (*circle one*): Cobble Wood Leaf Litter Fine Sediments Composite SAMPLE #: _____-____ __-____

Functional Group	Tally	Total	Identifiable Taxa
Shredders			
Scrapers			
Filtering Collectors			
Gathering Collectors			
Predators			

Sample Type (*circle one*): Cobble Wood Leaf Litter Fine Sediments Composite SAMPLE #: _____-_____-____

Functional Group	Tally	Total	Identifiable Taxa
Shredders			
Scrapers			
Filtering Collectors			
Gathering Collectors			
Predators			

in these patterns resulting from human impact (Cummins 1992, 1993). The objective of this chapter is to demonstrate how FFG ratios can be used as surrogates for these aquatic ecosystem attributes and serve as a useful assessment of the ecological condition (health) of freshwater communities. To accomplish this goal, we describe how invertebrates are organized into FFG categories and develop a set of ratios that can serve as surrogates for ecosystem attributes appear (Table 25.2). These ratios can be compared to proposed threshold values for appropriate ecosystem attributes to produce a qualitative evaluation of stream ecosystem "health."

II. GENERAL DESIGN

The general procedure described here focuses on identifying key functions in stream macroinvertebrate communities that can be determined at successive levels of taxonomic resolution. The technique is particularly useful for macroinvertebrate groups for which the state of taxonomic knowledge is presently incomplete. For example, determination of community structure for many groups of macroinvertebrates is based on measures of *taxa* richness or diversity (i.e., some combination of ordinal, family, and generic identifications), *not* on species richness or diversity. However, FFG analysis enables the evaluation of macroinvertebrate communities at a range of levels of taxonomic resolution. This approach maximizes the ecological information obtained for the taxonomic effort expended. For example, determination of functional feeding groups in the Odonata (dragonflies and damselflies) is achieved by separation to order alone. In contrast, FFG assignment for some subfamilies of Chironomidae (midges) or genera of ephemerellid mayflies may require species identifications.

A. Site, Habitat, and Timing of Sampling

Sites should be selected to ensure that the basic habitats are covered: (1) coarse sediments of riffles (golf ball to bowling ball-sized cobbles), (2) accumulations of organic litter and small woody debris (handful amounts), (3) fine sediments in depositional zones (the upper 1–2 cm scooped off of at least $0.5\,m^2$), (4) rooted vascular macrophytes, and (5) large woody debris (LWD). Because the results are presented as dimensionless ratios, they are relatively independent of sample size.

   If some habitat types are not present, the compromise might be, for example, that any erosional habitat present may have to suffice or be replaced by LWD as the only stable habitat where scrapers and filtering collectors can acquire appropriate food. In many streams, rooted vascular plants are not present and LWD may be scarce or absent. The three habitats that normally capture the full range of FFGs are coarse and fine sediments and plant litter of riparian origin. If samples are treated separately, results can be weighted for % cover of each of the habitat types which naturally favor different functional groups (e.g., cobble — scrapers and filtering collectors; leaf litter — shredders; fine sediments — gathering collectors). This can be helpful in providing a more balanced view of the study reach, but often the FFG ratios by habitat vary little from analysis of composite samples (e.g., Cummins *et al.* 2005). However, it is important to point out that the many protocols that sample riffles only will not provide information on shredders, and therefore yield little or no insight into the linkage of the stream community with the riparian zone (e.g., Cummins *et al.* 1989, Cummins 2002).

B. Collection and Processing of Samples

All sampling can be accomplished using a D-frame net (see Chapter 20) or a simple kick-net. Quantitative samples can also be taken with a Surber or Hess sampler from small gravel and cobble streams or the modified kick-net (see Chapter 20). Provided that the seasonal sampling issue is addressed (see following), a net mesh size of 0.5 mm is adequate. The net is held downstream and below a cobble or leaf pack as it is lifted from the stream, or the net is used to scoop surface sediments (∼2 cm depth) from depositional habitats, or scraped over the surface of LWD. If rooted vascular plants represent a significant habitat, they are shaken vigorously in front of the net (Merritt *et al.* 1996). Sample processing is rapid and can be conducted streamside. The significant advantages of sorting live samples on the spot are that the live animals are more easily detected and they retain their colors and behaviors as opposed to preserved samples. After sorting and enumeration by functional group, the samples can always be preserved and returned to the laboratory for detailed taxonomic work.

Processing in the field can be accomplished by washing the sample from the net into a white enamel tray or plastic dish tub for sorting. Muffin tins or plastic ice cube trays work well to provide separate wells for members of each functional group as they are removed and classified. Small spatulas made with 1-mm Nitex® work well. These are made by cutting 1-cm square pieces of Nitex® and sealing the boarders with hot glue with a large drop on one side into which is inserted the point of a dissecting needle to form a handle. These "bug spatulas" are particularly useful for capturing mobile mayfly and stone fly nymphs and work far better than forceps. If the sample contains large numbers of small, very mobile invertebrates, such as baetid mayflies, it may be preferable to simply make a total count in the pan or tray with a hand tally after the other animals have been removed. If the invertebrate density in the sample is very high, it may be advisable to separate the sample into quarters for sorting. Usually, the total count of animals removed from a sample need not exceed 100 in order to obtain sufficient data to calculate the FFG ratios.

Individuals are removed, assigned to an FFG using the keys (Appendix 25.1) and placed in the appropriate well in the muffin tin or ice cube tray. After sorting and separation is complete, the number of individuals in each FFG is tallied and taxonomic notes are made on a field data sheet (see example in Table 25.2). Specimens then can be preserved in a Whirl-Pak® in 70% ethanol, labeled, and returned to the laboratory for detailed taxonomic identification as desired. The taxonomy can be done using such texts as Pennak (1989), and Thorp and Covich (1991), Merritt and Cummins (1996).

The seasonal timing of FFG sampling is critical, just as it is for any taxonomically based numerical study. It is important to sample when the greatest number of taxa are in feeding stages and are as large as possible. This means avoiding periods of maximum egg laying, hatching, and adult emergence. Because distinctive fall-winter and spring-summer communities of stream macroinvertebrates exist (Cummins *et al.* 1989, Maloney and Lamberti 1995, Swan and Palmer 2004), at least two samplings per year are required to adequately characterize the functional feeding groups. These seasonal differences may be driven by annual cycles in either temperature or precipitation. In general, the optimal sampling times are mid- to late summer, to characterize the spring-summer populations and late fall to late winter, to characterize the fall-winter populations (Cummins *et al.* 1989).

Biomass data, which are preferable to numerical data but more time-consuming to obtain, can be estimated in the field by measuring *biovolume*. A small graduated cylinder (5–10 mL) can be used to determine volumetric displacement. The specimens in each

functional group are added cumulatively to the graduated cylinder containing an initial known volume of water. The volume of water displaced by each FFG collection is recorded and ecosystem surrogate ratios can be calculated using these volumes without conversion to actual estimates of biomass. However, biomass can be estimated in the laboratory from measurements of specimen lengths using length-mass relationships (e.g., Smock 1980, Benke *et al.* 1999), as was done for the data in Table 25.4.

C. Functional Group Designations

The key that appears below (Appendix 25.1) emphasizes higher-level taxonomic separations that permit reliable categorization of functional feeding groups. The key is organized into two levels of resolution. The first level can be used in the field with a minimum of taxonomic skill, usually resulting in an accurate separation of 80–90% of the specimens collected. You may also use Appendix 20.1 to supplement the taxonomic identification. Although the second level can be accomplished in the field with live material, this often requires a hand lens and the accuracy is less than if the analysis is conducted in the laboratory. The second-level analysis may increase the resolution by 5–10% by categorizing those macroinvertebrates that either do not readily fit the first level grouping or are likely to be misclassified. For example, the organic case-bearing trichopteran *Brachycentrus*, which is a filtering collector, would be classified as a shredder under the first level of resolution analysis (Appendix 25.1, Key 2).

D. FFG Ratios as Surrogates for Ecosystem Attributes

Examples of FFG ratios used as indicators of stream ecosystem attributes are summarized in Table 25.3. The ratios shown can serve as indicators of the relative importance of stream autotrophy or heterotrophy, the size categories and relative amounts of coarse CPOM and FPOM in transport and in storage, and the stability of the channel. Examples of FFG ratios and their relationship to proposed thresholds, as well as interpretation of the predictions, are given in Table 25.4. The use of FFG ratios as indicators of stream ecosystem attributes has been documented previously (e.g., Vannote *et al.* 1980, Minshall *et al.* 1983, Merritt *et al.* 1996, 1999, 2002, Stone and Wallace 1998, Wagner 2001, Cummins *et al.* 2004, Andrade 2006). Because the FFG method is responsive to changes in food resource base (e.g., algae, litter, fine organics, prey), it is sensitive to both general and site-specific impacts on riparian zones and watershed land-use. For example, the localized input of a toxic effluent in the form of DOM from a paper mill might be a site-specific disturbance, while increased sediment or reduced litter inputs resulting from altered land-use would be more general.

Case study. The example shown in Table 25.4, for a second-order, heavily shaded, woodland stream in the Allegheny National Forest (Pennsylvania) includes ecosystem evaluations based on FFG ratios (Table 25.3). The FFG ratio that serves as a surrogate for the stream autotrophy/heterotrophy index, or P/R (P = daily gross primary production and R = daily total community respiration), indicates that the stream is distinctly heterotrophic (Tables 25.3 and 25.4; see also Chapters 28 and 31). That is, the in-stream biology is heavily dependent upon allochthonous organic matter from the riparian zone. The surrogate FFG P/R ratio reflects the low numbers and biomass of scrapers linked to periphyton primary production and the high abundance of shredders and collectors that use detritus of riparian origin as a food resource.

TABLE 25.3 Examples of Functional Feeding Group Ratios as Indicators of Stream Ecosystem Attributes.

ECOSYSTEM PARAMETER	SYMBOLS	FUNCTIONAL FEEDING GROUP RATIOS	GENERAL CRITERIA RATIO LEVELS[1]
Autotrophy to Heterotrophy Index or Gross Primary Production to Community Respiration Index	AUTO/HETERO or P/R	SCRAPERS to SHREDDERS + TOTAL COLLECTORS	Autotrophic >0.75
Coarse Particulate Organic Matter (CPOM) to Fine Particulate Organic Matter (FPOM) Index	CPOM/FPOM	SHREDDERS to TOTAL COLLECTORS	Normal Shredder Association Linked to Functioning Riparian System
			Fall-Winter >0.5
			Spring-Summer >0.25
FPOM in Transport (Suspended) to FPOM in Storage in Sediments (Deposited in Benthos)	TFPOM/BFPOM	FILTERING COLLECTORS to GATHERING COLLECTORS	FPOM Transport (in Suspension) Greater Than Normal Particulate Loading in Suspension >0.50
Substrate (Channel) Stability	STABLE CHANNEL	SCRAPERS + FILTERING COLLECTORS to SHREDDERS + GATHERING COLLECTORS	Stable Substrates (e.g., Bedrock, Boulders, Cobbles, Large Woody Debris) Plentiful >0.50
Top-Down Predator Control	TOP-DOWN CONTROL	PREDATORS to TOTAL ALL OTHER GROUPS	Normal Predator to Prey Balance 0.10–0.20

[1] *General ratio ranges given are for numerical or biomass data taken when most species are in mid-to late larval instars or aquatic adults (see discussion under field sampling).*

For collectors, FPOM food would be derived from shredder feeding on CPOM, (i.e., fragments or feces) or directly from the riparian soil-litter layer. Data in Table 25.4 are from July when significant numbers of shredders (Plecoptera: Peltoperlidae; Trichoptera: Lepidostomatidae) were present. The FFG surrogate ratio that reflects the availability of appropriately conditioned (meaning suitable as a food for shredders; see Chapter 30) CPOM relative to FPOM indicates that the system is a "spring-summer shredder stream" (Cummins *et al.* 1989, Cummins 2002). That is, the shredders are dependent upon litter that requires a long conditioning time, such as hemlock needles. This conditioning time, which can be up to eight months or more for conifer litter (Cummins *et al.* 1989), is

TABLE 25.4 Example of Functional Feeding Group Ratios as Indicators of Stream Ecosystem Attributes. Data from a second-order stream in the Allegheny National Forest, Pennsylvania, in July.

ECOSYSTEM PARAMETER	FUNCTIONAL FEEDING GROUP RATIOS	CALCULATED RATIOS		GENERAL CRITERIA RATIO LEVELS	EVALUATION
		NUMBERS	BIOMASS		
AUTO/HETERO or P/R	SCRAPERS to SHREDDERS + TOTAL COLLECTORS	0.34	0.24	Autotrophic >0.75	Heterotrophic site, dependent on allochthonous organic matter inputs
CPOM/FPOM	SHREDDERS to TOTAL COLLECTORS	0.32	0.64	Normal shredder association linked to functioning riparian system in summer >0.25	A summer shredder stream; species dependent mainly on slow processing rate of litter[1]
TFPOM/BFPOM	FILTERING COLLECTORS to GATHERING COLLECTORS	0.87	1.76	FPOM transport (in suspension) enriched unusual particulate loading) >0.50	High FPOM loading (presence of Philopotamidae indicates very fine FPOM)
STABLE CHANNEL	SCRAPERS + FILTERING COLLECTORS to SHREDDERS + GATHERING COLLECTORS	1.08	1.03	stable substrates (e.g., bedrock, boulders, cobbles, large woody debris) plentiful >0.50	Channel stability high with numerous attachment sites for macroinvertebrates
TOP-DOWN CONTROL	PREDATORS to TOTAL ALL OTHER GROUPS	0.22	0.14	Typical predator to prey balance 0.10–0.20	Typical predator to prey ratio

[1] For example, see Cummins et al. (1989)

the period required for the plant litter to be colonized by stream microbes, especially hyphomycete fungi (see Chapter 15), to render it a food resource useable by shredders (Grubbs and Cummins 1994, 1996, Cummins 2002). Realize, however, that this general model for seasonal shifts in shredder dominance related to the type and timing of riparian litter inputs and the in-stream conditioning times required for each riparian plant species was developed from data for the North American temperate zone (e.g., Cummins *et al.* 1989, Cummins 2002). However, the spring-summer alignment between shredder abundance and litter that requires long in-stream conditioning time likely is applicable to the tropics as well (e.g., Grubbs and Cummins 1996, Forsberg *et al.* 2001, Wantzen *et al.* 2002, Cummins *et al.* 2005).

The FFG surrogate ratio for the availability of FPOM in transport (suspended load) relative to that in the benthos (bed load) indicates the availability of an abundant, good quality fine particulate food supply for filtering collectors (Table 25.3). The surrogate ratio is consistent with the source of this FPOM being organic fragments derived from processed fall-winter litter and organic soils from the riparian zone. The FFG surrogate ratio for channel stability reflects the requirement of scrapers and filtering-collectors for nonshifting surfaces, as opposed to the gathering-collectors and shredders that occupy the interstices of sediments and litter accumulations. The ratio indicates the availability of stable surfaces (Table 25.3) and supports the conclusion that the FPOM was derived from natural riparian and stream processes and not altered land use.

The FFG ratio of predators to prey is in the expected range and indicates that there is a balance at the stream site between prey species with long and short life cycles (Table 25.4). That is, a high top-down ratio (>0.15) would reflect the dominance of prey species having short life cycles and, therefore, populations that turn over rapidly to continuously renew the food supply for the longer-lived predators.

Thus, in this example, the FFG ratios are consistent with the observations of the ecosystem properties at the sampling site and, by inference, this second-order stream as a whole. Additional examples of the use of FFG ratios as surrogates for these stream ecosystem attributes can be found in Merritt *et al.* (1996, 1999, 2002) for south Florida rivers and Cummins *et al.* (2005) for streams in southeast Brazil.

III. SPECIFIC METHODS

A. Basic Method 1: Determining Macroinvertebrate Functional Feeding Groups in the Field

1. Establish sampling teams of two or three individuals.
2. Each team should take at least one sample from each of the three general habitat types (rock, litter, fine sediments) using a D-frame or large aquarium net. If the habitats are to be evaluated independently, the samples should be analyzed separately and a reach-scale evaluation performed by combining the data later. For a faster, but less detailed exercise that provides a reach estimate only, the samples from the habitats can be combined before sorting. If other habitats are in evidence (e.g., rooted macrophytes, large woody debris), they should also be sampled or included in a composite sample. The minimal goal is to have each team produce three FFG ratios, one for each habitat type, or one ratio if habitat samples are combined before sorting.
3. Special care is required when sampling cobble from riffles to note any caddisfly (Trichoptera) nets that are present. The nets are diagnostic for some caddisfly

larvae but are destroyed during sampling and the larvae will be moving about freely and can be confused with non-net spinning, free-ranging predaceous caddisfly larvae (family Rhyacophilidae).

4. Wash the sample to be sorted (i.e., invertebrates to be removed) into the white enamel tray or plastic dish tub. Ensure that the sample is covered with 2 to 4 cm of water. Leaves, large pieces of wood, cobble, and gravel can be washed off into the tray with a squeeze bottle to remove invertebrates and discarded to facilitate sorting. If the sample is to be returned to the laboratory for analysis, transfer it to a Whirl-Pak® bag containing a label (pencil on sturdy white paper) with sampling team designation, date, site, habitat or composite, and an identification number that can be referenced to any field notes taken. Preserve the sample with 70% ethanol (if samples contain significant water, then a higher concentration of ethanol may be needed). Skip to the "Laboratory Analysis" section below.

5. Remove invertebrates from the sample with the bug spatula described above and sort by functional group first-level of resolution, using the FFG key (Appendix 25.1), into separate wells of the muffin tin or ice cube tray for enumeration.

6. Total the number of organisms in each FFG (this will be facilitated by the use of a hand counter; see example in Table 25.2) and calculate the FFG ratios for each of the habitat types, or for the total if the sample is a composite, following the format shown in Table 25.3.

7. Interpret the ratio data relative to the expected threshold levels (Table 25.3) as shown in the example in Table 25.4. This allows the ecosystem condition for each habitat or for the reach as a whole to be evaluated from a composite sample or by combining habitats. If there are multiple teams, all data should be combined and means calculated for each habitat and reach to allow for a general assessment of ecosystem condition using data from all teams. See Chapters 2 and 35 if more extensive physical habitat assessment is to be combined with the FFG analysis.

B. Advanced Method 1: Optional Field Exercise

1. After steps 1–5 above, determine the biovolume of each functional group (i.e., a composite of all individuals in each group) using a graduated cylinder. This transfer will be facilitated by the use of fine-point (jeweler's) forceps.

2. Complete steps 6 and 7 from Basic Method 1 using volume measures as well as the numerical data.

3. Evaluate the differences in the ratios and assessments of ecosystem condition when numerical and biovolume data are compared.

C. Basic Method 2: Determining Macroinvertebrate Functional Feeding Groups in the Laboratory

1. If samples are taken and preserved in the field, as described in step 4 of the field procedure above, empty the contents of a Whirl-Pak® into a petri dish.

2. Remove invertebrates from the sample and assign them to FFGs using a dissecting microscope. Use both levels of resolution in the FFG Key (Appendix 25.1). Then identify specimens in each FFG to genus, or lowest possible taxonomic level, using Pennak (1989), and Thorp and Covich (1991), Merritt and Cummins (1996). The

ecological tables in Merritt and Cummins (1996), and text descriptions in Pennak (1989), should be used to further refine the functional group separations.

3.   Complete steps 6 and 7 from Basic Method 1.

D. Advanced Method 2: Optional Laboratory Approach

1.   After the specimens are sorted, identified, and categorized into FFG in the laboratory, measure and record the length of each specimen to the nearest mm under a dissecting microscope, using a clear plastic mm rule placed on the microscope stage under the petri dish, or with a calibrated ocular micrometer.
2.   Convert the length of each specimen to an estimate of its dry biomass using the regression equations in Smock (1980) and Benke *et al.* (1999).
3.   Complete steps 6 and 7 from Basic Method 1 using the calculated dry biomass data.

IV. QUESTIONS

1.   How could the timing (i.e., season) of your sampling have influenced your estimates of FFG ecosystem surrogate ratios and the interpretation of ecosystem conditions?
2.   When and why would FFG ratios calculated on the basis of biomass yield a better estimate of FFG invertebrate community structure than numbers of individuals?
3.   How would community structure, as indicated by FFGs, be influenced by changes in riparian cover? For example, how would an increase in incident radiation reaching the stream and loss of litter inputs affect FFG ratios?
4.   Would FFG analysis be a more useful metric than a diversity index in assessing the effects of sewage effluent on a stream macroinvertebrate community? If not, why?
5.   In the analysis of the selected stream, which group of collectors (filtering or gathering) was most abundant? Speculate as to why.
6.   What are some of the advantages and disadvantages of the use of FFG ratios, as compared to other aggregate measures such as diversity indices or the index of biotic integrity? Why not just use individual taxonomic identifications?
7.   Many macroinvertebrate rapid bioassessment methods specify riffle sampling only. Considering the focus of the FFG method, what would be missed by such an approach?

V. MATERIALS AND SUPPLIES

Field Materials and Supplies

> 95% ethanol
> Hand calculator
> White enamel sorting trays or plastic dish tubs
> "Bug spatulas" (see above)
> Fine-point (jeweler's) forceps
> Graduated cylinders (1, 5, 10 mL, graduated in 0.01 to 0.1 mL divisions)
> Hand lens
> Hand tally counter

D-frame or large aquarium net
Multiple-compartment container (muffin tins or ice cube trays) for
  separating specimens into FFGs before counting.
Whirl-Pak® bags

Laboratory Materials and Equipment

Dissecting microscope
Clear plastic ruler (mm graduations) or ocular micrometer
Petri dishes
Taxonomic guides (e.g., Pennak 1989, and Thorp and Covich 1991, Merritt and
  Cummins 1996).

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APPENDIX 25.1
A Simplified Key to the Functional Feeding Groups of Lotic Macroinvertebrates

KEY TO FUNCTIONAL FEEDING GROUPS

⊢ ⊢────── Indicates size or range of sizes

1. ANIMALS IN HARD SHELL (Phylum Mollusca)
   a. LIMPETS (Class Gastropoda)
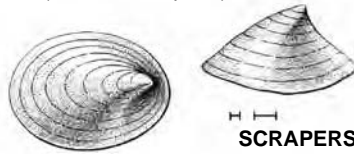
SCRAPERS

   b. SNAILS (Class Gastropoda)
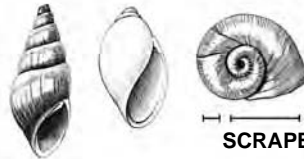
SCRAPERS

Snails are generalized (facultative) feeders
and can also function as Shredders.

   c. CLAMS OR MUSSELS (Class Pelecypoda)
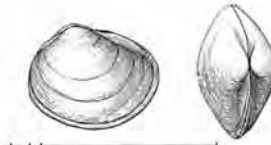
FILTERING COLLECTORS

2. SHRIMPLIKE ANIMALS (Class Crustacea)

Decapoda   Isopoda   **SHREDDERS**   Amphipoda
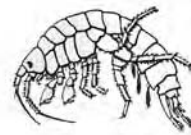
Can also function as facultative Gathering Collectors.

3. LARVAE IN PORTABLE CASE OR "HOUSE"   Go to KEY 2

4. LARVAE IN FIXED RETREAT WITH CAPTURE NET   Go to KEY 3
   Note: Care must be taken when collecting to observe nets.

5. WITHOUT CASE OR FIXED RETREAT

   a. WORMLIKE LARVAE, WITHOUT JOINTED LEGS   Go to KEY 4

   b. NYMPHS OR ADULTS WITH JOINTED LEGS   Go to KEY 5

6. DOES NOT FIT KEY 5 EXACTLY   Go to KEY 6
                                                or KEY 7

KEY 2

FIRST LEVEL OF RESOLUTION

LARVAE IN PORTABLE CASE
Caddisflies (Order Trichoptera)

CASES ORGANIC
Leaf, stick, needle, bark

CASES MINERAL
Sand, fine gravel
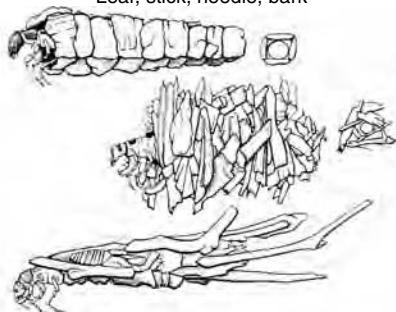
Families Limnephilidae (in part)
Lepidostomatidae (in part),
Phyrganeidae, Leptoceridae (in part)

SHREDDERS
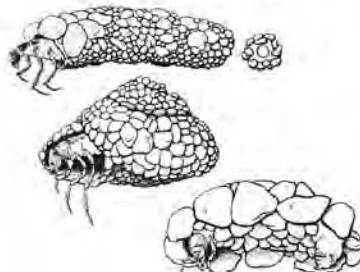
Families Glossosomatidae,
Limnephilidae (in part),
Helicopsychidae

SCRAPERS

SECOND LEVEL OF RESOLUTION considers a few fairly common caddisflies that would be misclassified above on the basis of case composition alone.

CASES ORGANIC

CASES MINERAL

Cases square in cross section and tapered, with no bark or flat leaf pieces included. Front attached to substrate. Larvae extend legs and filter the current

Cases long, slender, and tapered, made of plant material

Cases long, slender, and tapered (mostly fine sand) or cases ovoid and very flat in cross section

Foreleg with filtering hairs

Family Brachycentridae (in part)

Family Leptoceridae (in part)

Family Leptoceridae (in part)
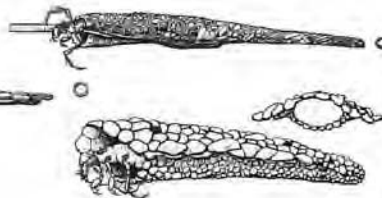
FILTERING COLLECTORS

GATHERING COLLECTORS

GATHERING COLLECTORS

KEY 3

FIRST LEVEL OF RESOLUTION

LARVAE WITH FIXED RETREAT AND CAPTURE NET
Note: Care must be taken when collecting to observe nets.

Caddisflies (Order Trichoptera)

True Flies (Order Diptera)

COARSE NET IN "SCAFFOLDING"

FLATTENED SOCKLIKE OR TRUMPET-SHAPED NET OF FINE MESH

TUBE WITH SILK STRANDS STRUNG BETWEEN TERMINAL PRONGS

True Midges (Family Chironomidae)

Families Hydropsychidae, Philopotamidae, Polycentropodidae

FILTERING COLLECTORS

SECOND LEVEL OF RESOLUTION separates from free-living larvae those net-spinning caddisflies that may have been inadvertently collected without being associated with their nets.

NET-SPINNING CADDISFLIES
Frequently separated from their nets

FREE-LIVING CADDISFLIES
Non net-spinning

HEAD AS WIDE AS THORAX
Especially Philopotamidae (bright yellow) and Hydropsychidae(bright green or brown)

HEAD LONG, SMALL, AND NARROWER THAN THORAX

Rhyacophilidae (often bright green)

FILTERING COLLECTORS

PREDATORS

KEY 4

FIRST LEVEL OF RESOLUTION

WORM-LIKE LARVAE WITHOUT JOINTED LEGS

LARGE

SMALL

Larger than |————————|

smaller than |————|

Head retractile and poorly developed.
Caudal lobes with eyelike spiracles

LONG AND SLENDER

BOWLING PIN SHAPE

Bulbous base usually fastened tightly
to substrate

Craneflies (Family Tipulidae (in part))

SHREDDERS

True Midges (Family Chironomidae)

Note: Subtract 10% of count for Predators

Blackflies (Family Simuliidae)

GATHERING COLLECTORS

FILTERING COLLECTORS

SECOND LEVEL OF RESOLUTION considers some wormlike Predators that would be misclassified in the above key.

WORM-LIKE LARVAE WITHOUT
JOINTED LEGS

LARGE

SMALL

Posterior segment swollen.
Head retractile

Prolegs along entire length.
Head visible

Prolegs poorlydeveloped
or absent; jaws well
developed; very active

Family Tipulidae (*Eriocera* type)

Family Athericidae

Family Tipulidae (*Dicranota* type)

PREDATORS

KEY 5

FIRST LEVEL OF RESOLUTION

NYMPHS WITH JOINTED LEGS

3 (or 2) TAILS (FILAMENTS) AT BACK.
NO EXTENDIBLE LOWER LIP (LABIUM)

3 FLAT PADDLES OR POINTS AT BACK.
EXTENDIBLE LOWER LIP

3 (or 2) TAILS WITH LATERAL
ABDOMINAL GILLS
Mayflies (Order Ephemeroptera)

2 TAILS WITHOUT LATERAL
ABDOMINAL GILLS
Stoneflies (Order Plecoptera)

3 FLAT PADDLES AT BACK

POINTS AT BACK

Body shape ovoid.
Flat in cross section

Body shape cylindrical.
Round in cross section

Bright color pattern.
Very active

Dull brown or black.
Sluggish

Damselflies
(Suborder Zygoptera)

Dragonflies
(Suborder Anisoptera)
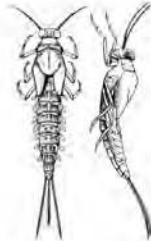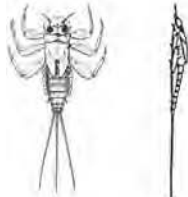
Families Heptageniidae,
Ephemerellidae (in part)

Families Baetidae, Leptophlebiidae,
Ephermerellidae (in part), Ephemeridae

Setipalpian Stoneflies

Filipalpian Stoneflies

PREDATORS

PREDATORS

SCRAPERS

**GATHERING
COLLECTORS**

PREDATORS

SHREDDERS

KEY 6

SECOND LEVEL OF RESOLUTION considers some fairly common insects that do not fit in the above key or would be misclassified on the basis of body shape alone.

LARVAE, NYMPHS, OR ADULTS WITH JOINTED LEGS. WITHOUT CASE OR FIXED RETREAT

WITH LONG TAIL(S) OR CAUDAL HOOKS

WITHOUT LONG TAILS
Beetles (Order Coleoptera)

CAUDAL HOOKS

1 TAIL (FILAMENT)

3 LONG CERCI

Body shape ovoid. Rear end often erected scorpion-like when disturbed

Body shape cylindrical. Long hairs on inside of front legs

With ventral suckers or disc shape. Head and legs totally concealed beneath

Adult beetle

Larvae slender, triangular in cross section

Family Corydalidae

Family Sialidae

Family Ephemerellidae (in part), Caenidae, Tricorythidae

GATHERING COLLECTORS

Family Isonychiidae

FILTERING COLLECTORS

Water Pennies (Family Psephenidae)

(Order Diptera) Lobed body. Ventral suckers

Family Blephariceridae

SCRAPERS

Riffle Beetles (Family Elmidae)

SCRAPERS

If larva has spines along lateral margins, it is a wood-eating Shredder

Riffle Beetles (Family Elmidae)

GATHERING COLLECTORS

PREDATORS

KEY 7

SECOND LEVEL OF RESOLUTION considers some fairly common insects that do not fit in the above key or would be misclassified on the basis of body shape alone.

NYMPHS AND ADULTS
WITH BEAK AND HEMELYTRA

Beak triangular,
front legs smaller

Sharp beak,
front legs large

Family Naucoridae

Family Corixidae

SCRAPERS

Family Belostomatidae

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Trophic Relations of Stream Fishes

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I. INTRODUCTION

Conceptually, trophic relations of fishes begin with foods and feeding behavior of individuals or species, but also include feeding guilds (i.e., species or life-stages with similar morphology or using similar foods or feeding behaviors; see also Chapter 22) and food webs (see Chapter 27). Descriptions of food use by individual species are compiled in review volumes (Carlander 1969, 1977, 1997, Gerking 1994, Matthews 1998), state references (Robison and Buchanan 1984, Jenkins and Burkhead 1994, Etnier and Starnes 2001), and workshop proceedings (Stouder *et al.* 1994). Viewed across all species and life-stages, fishes feed on many types of organisms and, in turn, are food for many aquatic and terrestrial predators, including humans. Thus, trophic interactions of fishes forge links (direct and indirect) throughout aquatic, and even into terrestrial webs (see Chapter 27). The position of an organism within a food web is largely defined by its fractional trophic level (Pauly and Christensen 2000), which is generally positively related to body size (Cohen *et al.* 1993) and allometric growth because mouth size and shape often determine the types of prey consumed (Keast and Webb 1966, Werner and Hall 1979, Norton 1991). Therefore, relationships among morphological characteristics are of paramount importance when quantifying feeding patterns and defining the ecological role of organisms within food webs (see Chapter 27). In systems where the principal route of ecosystem energy flux and material cycling is through detrital processing (Bowen 1983; Catella and Petrere 1996), greater importance of detritus in fish diets is considered a strategy for

shortening food chains, and thus, increasing community efficiency (Vaz *et al.* 1999). Such studies usually focus on variation of internal anatomy.

Competition for prey, although more difficult to demonstrate than predation itself, can structure communities across ecological and evolutionary time scales. Under relatively predictable conditions, expectations from ecological niche theory are that species will exhibit some type of resource partitioning in order to minimize competitive interactions (Hutchinson 1959). Therefore, fish species might partition both resources produced within the stream (i.e., autochthonous) and resources that enter the food web from riparian zones outside of the stream (i.e., allochthonous). Moreover, differences in timing of supply and size range of prey can drive selective foraging by fishes (Nakano *et al.* 1999). However, when interactions between native and introduced species do not result in niche shifts, a behaviorally dominant, introduced species is expected to out-compete the native (Gunckel *et al.* 2002). The degree of trophic specialization can influence overall system persistence by altering the ecological composition and indirect effects among community members (Huxel *et al.* 2002).

Due to indeterminate growth, a fish can function as multiple 'ecological' species across the (ontogenetic) stages of its life. This is primarily attributed to ontogenetic changes in mouth dimensions, visual acuity, digestive capacity and swimming performance, which, among other factors, allow growing predators to successfully ingest larger prey (Keast and Webb 1966, Werner and Gilliam 1984). Indeed, body form and mouth size are among the most important factors determining fish diet (Breck 1993, Magnhagen and Heibo 2001), and morphological changes over time affect foraging ability and differential exploitation of food resources (Castro and Hernández-Garcia 1995, Wainwright and Richard 1995, Magnhagen and Heibo 2001). Prey morphology also undergoes ontogenetic change, so as predator gape size changes, their morphological defenses become more (or less) effective (Timmerman *et al.* 2000, Hjelm and Johansson 2003). Therefore, even within a single predator population (especially among individuals in their first year of life), divergent morphological characteristics due to differential survival are congruent with genotype, or phenotypic plasticity. However, if older fish move long distances within and among river basins (e.g., during reproductive migrations), such links between ecological and evolutionary forces are weakened.

In as much as trophic studies can help explain recruitment dynamics within a species and across various habitats (Cutwa and Turingana 2000), they are valuable in making decisions related to natural resources management (Kido 1996). Diet analysis can help quantify the threat of an introduced fish species to population dynamics as well as trophic interactions in the system, as when a native keystone predator is threatened with replacement by a nonnative predator, either through competition for prey or direct predation on offspring of the native species, or both (Fletcher 1991, Fritts and Pearsons 2004). Diet studies help to assess ecosystem integrity and assemblage functional redundancy (Matthews *et al.* 1982; see also Chapter 22).

The association between a fish's food resources and its morphological traits (i.e., a phenotype-environment relationship) together with effects on feeding performance and growth suggest mechanisms for adaptive radiation (Schluter 2000). Studies of the evolution of trophic adaptations have used field and laboratory studies to test the accuracy of predictions about species interactions (DeWitt and Scheiner 2004), and their consequences for management (e.g., Polis *et al.* 1989, Kido 1996, Olden *et al.* 2004). Intraspecific resource polymorphism (i.e., a single species displaying forms with differential niche use) in vertebrates might be common and usually is associated with a perceived "open" niche (Robinson and Wilson 1994, Wimberger 1994, Skúlason and Smith 1995). Most of these

have been in organisms such as birds and fish that subdue, manipulate, and process their prey, using only their mouth (Wimberger 1994). In fishes, resource polymorphism arises from habitat-specific foraging (Robinson and Schluter 2000), but studies primarily focus on end stages of ontogeny (but see Ehlinger and Wilson 1988, Wainwright *et al.* 1991, Robinson *et al.* 1996), despite the rich variation across life stages (Werner and Gilliam 1984). Much variation derives from scaling constraints on foraging performance and energy demands (Persson *et al.* 2000). During ontogeny, fish are subjected to different, even conflicting, selection pressures, which reduced performance in comparison to organisms that specialize in one niche throughout development (Werner and Gilliam 1984).

This chapter provides tools to generate and test hypotheses about trophic relations of fishes and their likely effects on ecological and evolutionary processes. We include basic and advanced methods currently used for collecting and analyzing data designed to answer questions such as (1) What, when, where, and how much does a fish eat? (2) What trade-offs are involved in trophic interactions that shape these choices (including the risk of being eaten by its own predators)? (3) What adaptations to resource conditions (e.g., Robinson and Wilson 1994; Mittelbach *et al.* 1999) are subsequently expected? Methods focus on ambient interactions, but researchers are encouraged to recognize the relationships to natural selection and extant phylogenetic differences, especially when designing research programs. For example, interspecific studies of the relationship between morphological and dietary variables can result in dubious correlations if such studies disregard variance due to shared ancestry (Felsenstein 1988, Westneat 1995). We suggest experimental manipulations and reference advanced methods that extend basic studies by combining them with behavioral and biochemical methods, and discuss the suitability of various analyses.

II. GENERAL DESIGN

There are two basic approaches to understanding trophic relationships. First, a mechanistic approach focuses on function. For example, to understand what factors influence a fish's diet, one might study the behavior of a fish as it first detects, and then handles, and finally eats its prey. One might further explore the consequences to the individual fish (e.g., what components of the prey are incorporated into the fish's own tissues and the effects on growth, or how soon the fish will eat again, and if it will be the same for a different prey item). Such data can link proximate functional performance of individuals to community dynamics, and ultimate evolutionary outcomes (DeWitt and Langerhans 2002, Ferry-Graham *et al.* 2002). Second, in a theoretical approach, one might model an optimal set of conditions and choice of prey intended to maximize the fish's ecological fitness as measured by gains and losses (profitability) to the fish, measured as energy and time (Hughes 1997). These characteristics should be considered in designing tests of diet choice, especially if the aim is to correctly predict occurrence and success of real interactions (Sih and Christensen 2001) or to quantify losses of important prey species due to predation and subsequent population effects (Fritts and Pearsons 2004).

Field Studies of Fishes

Field studies of diet, morphology, and behavior can be used to extend results from the methods described below. Use methods from Sections C and D of this book to place your results within the context of environmental conditions, habitat, other biota, and

community interactions. For example, actual prey consumption can be compared to prey availability (estimated from collections of stream biota; see Chapter 20) and used to calculate an index of trophic specialization, or to compare congruence between diet and morphology of fishes in target and reference communities (e.g., least-disturbed streams) to indicate relative integrity of the stream ecosystem. During floods, increased turbidity and suspended debris in the water column reduce the search area and capture efficiency of feeding juvenile salmonids (Berg and Northcote 1985, O'Brien and Showalter 1993). Territorial behavior might break down at such times, as detected by decreased variability in individual growth when compared to base-flow conditions (Berg and Northcote 1985). Combine observational and manipulative field experiments (Hughes *et al.* 2003) to test models of trophic behavior and expected outcomes for stream food web structure and function (see Chapters 22 and 27). Fences made with steel T-posts and polyethylene plastic mesh (5 mm, commonly used in aquaculture) placed across riffles, or configured as pens within pools, allow invertebrates to pass through, yet selectively prevent passage by fishes (Gelwick and Matthews 1992).

Mesocosm Studies Using Fishes

Mesocosm studies combined with field studies can enhance not only the statistical power of experiments, but also inference about structure and function of natural systems (Gido *et al.* 1999, Evans-White *et al.* 2001, Gido and Matthews 2001). The significance of behavior has been inferred largely from field collections that infer differences in foraging morphology and diet, rather than direct observation (Robinson and Wilson 1994, Robinson *et al.* 1996). Particularly when behavior of fishes is difficult to observe under natural conditions, complementary studies using mesocosms, in which individual fish can be observed, improve our understanding of how behavior produces and maintains resource polymorphisms. Modular systems of experimental pools and riffles (Gelwick and Matthews 1993) have been used for experiments lasting up to 14 months and have involved a combined total of >30 fish species, which exhibited normal body condition (as compared to our observations of these species in natural streams) and behaviors, including reproduction. One configuration used to test effects of increased temperature on fish community interactions is shown in Figure 26.1 (see also Figure 3 in Lamberti and Steinman 1993). Yet, even as one gains experimental control by using such systems, reality of scale challenges experimental design, especially regarding through-flow, nutrient cycling, and invertebrate colonization and drift. By addressing such challenges we come to a greater understanding of the natural system.

Diet and Morphology

Diet and related (trophic) morphological characteristics often correlate with resource use (Keast and Webb 1966, Gatz 1981, Mittelbach *et al.* 1992), but accuracy should not be assumed without testing the relevance of morphological abilities (or constraints) to the organism in its specific environment (Douglas and Matthews 1992, Shoup and Hill 1997). If size distribution among competing individuals fluctuates among years, then natural selection should favor genotypes with phenotypic plasticity that is determined by ambient resource availability (Mittelbach *et al.* 1992, Langerhans *et al.* 2003). Models of population dynamics explore the importance of size structure to expression of different phenotypes and ontogenic trajectories, and conversely the effect of phenotypic variation on dynamic patterns in population structure (Hjelm and Johansson 2003).
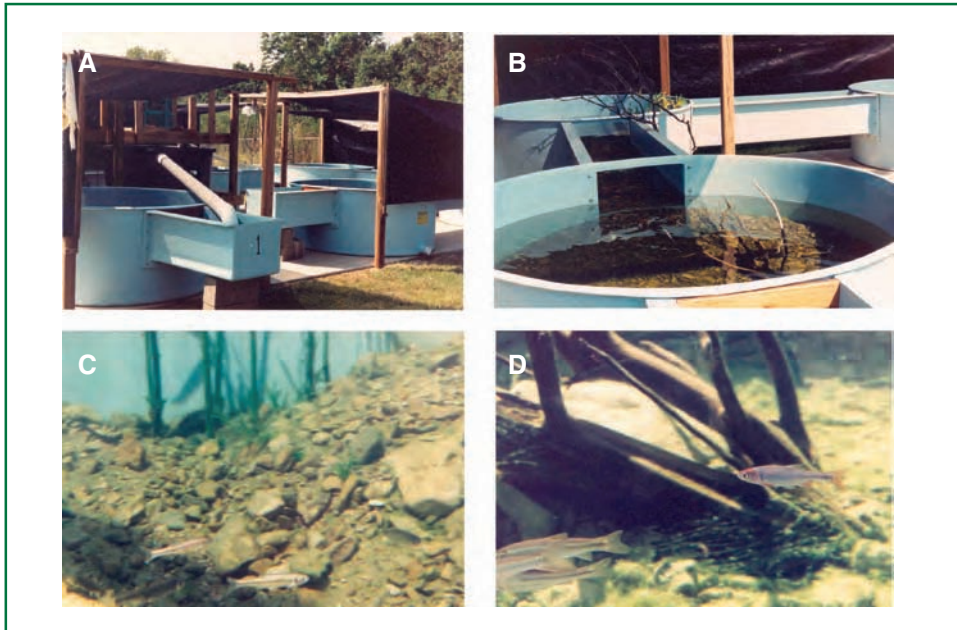
FIGURE 26.1 Experimental streams at University of Oklahoma Biological Station (A: overview; B: "riffle-pool" couplet) and view through side windows of fish use of habitat types inside pools (C: gravel in pool; D: artificial structure).

Most predatory fishes eat whole prey items, leading to a size range in prey from those too small for visual detection or physical retention by the trophic apparatus (e.g., gill rakers), to those too large to pass into the digestive system (e.g., between the bones of the jaw or the paired cleithra that surround the opening of the esophagus; Lawrence 1958, Timmerman *et al.* 2000). Allometric changes in trophic structures generally widen the range of potential prey as a fish grows, but differently among species. Thus, size range of available prey sets conditions under which to evaluate behavioral changes, growth, and recruitment.

A fish can eat individual small prey having low handling time but with little energy reward or eat larger prey to improve energy intake but incur a greater time cost, the effect of which gains significance as prey get larger. The trade-off of keeping costs low while striving for maximum reward is central to many feeding studies (Nilsson and Bronmark 2000, Delariva and Agostinho 2001). Food in the gut also affects behavior in relation to energy reserves. Feeding motivation is closely linked to satiation, which signals animals to regulate their food intake. If larger prey size increases handling time, it may also reduce capture success rate, causing fish to choose alternate (more profitable) prey (Juanes 1994). Feeding on relatively smaller prey should change both capture success and stomach fullness more gradually, and hungry fish are generally less choosey (Gill 2003). These are important considerations in building foraging models that predict prey choice (Hirvonen and Ranta 1996, Clark and Mangel 2000). Alternatively, complete fullness may not always be optimal as when other factors become more important and predators benefit from experience with various prey. In experiments or models, piscivore diet usually reflects feeding success during simultaneous encounters with prey of different vulnerabilities (Juanes 1994). However, for drift-feeding fishes in natural systems, the importance of

prey density or encounter rate is reflected in the fly fisher's mantra to "match the hatch." Fish feed first on the most dense prey swarm, then change to lower-density prey as their stomach fills. This suggests a trade-off between feeding and vigilance; high prey densities provide food at a faster rate but reduce the fish's ability to detect its own predators.

Little attention has been paid to the combined effects of behavior, diet, and morphology on variation in growth among individuals of the same fish population, despite large variations in size at age. Size differences can cause individuals to move into poorer quality habitats having reduced feeding opportunities (Rincón and Grossman 2001). Given the high mortalities that fish incur over their first growing season, one could reasonably expect lower variation in any flexible trait as age and size increase. Activity costs can be an important component of the energy budget of drift-feeding fish, and variance in their growth rate (Boisclair and Leggett 1988). Resource polymorphisms are attractive research subjects because they offer an opportunity to examine the role of ecological interactions (e.g., competition and resource partitioning) in evolutionary processes. Thus, scales of spatial and temporal experiments should also be relevant to such short-term variation as that experienced by juvenile fish, especially when linked to recruitment (Arndt *et al.* 2002). Moreover, the response rate (sensitivity) of a given measurement to change in nutritional status of individual fish, should match the sampling regime (e.g., RNA:DNA ratios versus fat content).

Data Collection, Synthesis, and Analysis

These elements are important considerations in feeding studies. Behavioral observations, laboratory procedures, and data compilation and analysis can be carried out entirely by one individual, a few trained individuals, or allocated among groups in order to isolate and reduce measurement error. Active gears, such as electrofishing, beach seines, and trawls are used for representative samples at regular intervals over 24-h periods. For large fish that can evade active gear, passive gear (e.g., gill nets) is usually set over a similar time period, but retrieved more frequently (hours). However, exact time of entanglement for each fish is generally unknowable, and if catches are combined into successive intervals, this temporal variability and uncertainty to conclusions (Cortés *et al.* 1996). Feeding activity is constrained by environment, behavior, and physiology. So one should also consider the percentage of empty stomachs in a sample, as well as composition and state of digestion of each prey in each fish gut at each sampling interval. If the digestion rate of different prey items is known, then meal sizes and ingestion times can be reconstructed.

III. SPECIFIC METHODS

A. Basic Method 1: Analysis of Fish Gut Contents Using Dissection

In this method, you will obtain and preserve fish in the field and then perform fish dissection, which allows the gut to be examined for fullness, all prey to be removed and measured, and the morphology of trophic structures to be studied (see *Basic Method 3* following). In the laboratory, gut contents will be identified and quantified for relative abundance of different prey items.

Field Procedures

1. In the field, teams of three researchers will allow two to manipulate sampling gear and one to carry a container for fish and attend to instruments and data. Multiple teams should stay separated by at least one habitat unit (e.g., pool, run, or riffle) so as to minimize interference.
2. Sample specific habitats with appropriate combinations of gear (see Chapter 22). This allows fish to be chosen from a particular size or range of size classes (e.g., ontogenetic studies) and species (e.g., piscivores and their prey). A suction device (e.g., slurp gun) can be used to collect schools of larval fishes (<12 mm). Larger postlarvae and juveniles can be collected using small dip nets or seines.
3. Place whole fish into plastic jars (pre-label or put a label inside) one-third full of ice-water to immobilize them, and then euthanize them in lethal solutions of either Clove Oil or MS-222 (see Chapter 22). Once euthanized, preserve specimens in 10% formalin (see Chapter 22 for warnings about the use of formalin and MS-222). Fish >100 g should be injected with formalin into the gut area to prevent further digestion of prey (see alternative preservation methods).
4. *Alternative preservation method:* All materials (whole fish, excised gut contents) that are not chemically preserved should be returned to the laboratory on ice, and then stored frozen until they are thawed and examined. Formalin-preserved fish and stomach contents are rinsed with fresh water, and then stored in 70% ethyl alcohol. Alternatively, piscivore gut contents can be preserved in saturated sodium bicarbonate solution, and whole fish placed on dry ice (Note: observe federal and state safety requirements when shipping) for longer storage (one to three months; Fritts and Pearsons 2004). Note that manipulations of specimens for some morphological measurements (e.g., mouth diameter and protrusability, gut length) are difficult on rigidly preserved specimens, compared to fresh tissues. Alternative methods of anesthesia, restraint, and preservation are given in the *Guidelines for the Use of Fishes in Research* (American Fisheries Society *et al.* 2004).

Laboratory Procedure: Obtaining Gut Contents

Fish gastrointestinal tracts can be divided into foregut (if a true muscular stomach is not present, this is the section anterior to first bend) and hindgut (intestine) regions. Individual fish having nothing in their foregut can either be categorized as empty, or if food is present in the anterior 5% of the intestine, prey can be identified and noted as an alternative record.

1. Using appropriately sized scissors or scalpel, make a longitudinal cut on the ventral side of the fish from just behind the isthmus of the gills posterior to the anal fin. Then make two transverse cuts at each end of the first cut to open the coelom and expose the viscera. Using sharp scissors, sever the esophagus, the last few mm of the intestine, and the mesentery at its dorsal point of attachment. This allows the visceral mass to be lifted out of the coelom for more detailed examination and manipulation.
2. Separate the digestive tract from the other visceral organs. Note the presence of a coiled gut (e.g., herbivores) or gastric caecae (fingerlike projections from the stomach of piscivores), or conduct alternative morphological measurements.

3. Sever the stomach (or foregut) from the hindgut. This section will contain the most recently ingested prey. Foreguts can be weighed with contents and then total content wet mass calculated by subtraction of empty gut weight.

4. Open the stomach or gut segment by making a shallow slit (be careful to not cut prey) lengthwise with fine scissors. For piscivores, whole prey items can be lifted directly from the stomach. For smaller prey, it is often useful to hold the slit segment with forceps over a petri dish and wash out the contents with a small amount of water from a squirt bottle or pipette. The food also can be extruded by sliding a blunt probe along the length of the segment; this may extrude much of the gut mucosa as well, which should not be mistaken as part of the diet (Bowen 1983)

    a. To identify prey of piscivores based on bone morphology, immerse gut contents of the piscivorous fish in a porcine pancreatin solution consisting of 1 g pancreatin powder, 65 mL lukewarm tap water, and 35 mL saturated borate solution (buffer).

    b. Place separate samples in a drying oven at 40°C for 2 to 24 h, depending on the size of the fish.

    c. Place a single stomach sample into a petri dish; count and identify prey fish to the lowest possible taxon based on diagnostic bones (refer to published keys and sketches such as Hansel *et al.* 1988).

Laboratory Procedure: Identifying and Quantifying Gut Contents

Gut contents are best viewed using stereoscopic dissection and compound light microscopes. Various metrics provide different insights into feeding habits. Prey can be quantified simply by occurrence (i.e., present or absent in each individual fish), which as an aggregate indicates habits of the sampled fish population. Counts, volume, and mass (Hyslop 1980) help to quantify feeding behavior, bioenergetics, and nutritional value. The approach depends first on whether or not discrete items can be identified and counted. Algae and detritus (fine organic material in various stages of decomposition) are better estimated as area or volume (e.g., points method, where items are examined in a counting chamber under a stereomicroscope, and the area occupied by each item evaluated relative to total area of all prey combined; Hynes 1950).

Even recently ingested food items may be ground by pharyngeal jaws and teeth (Figure 26.2C and D), making recognition difficult; digestion-resistant parts of prey (head capsules and shields, tarsal claws) are most useful. We highly recommend that a reference collection be made at the stream site to aid prey identification, especially for benthic macroinvertebrates (see Chapter 20). In addition, other potential food items such as vascular plants can have characteristic leaf shapes or edges (see Chapter 18) and algal cells often remain intact (see Chapter 16). The level of prey identification should be determined by the researcher's skill, time available and information needed. Functional categories of prey can be assigned based on the apparent behavioral and functional challenges that the predator overcame in order to capture and process prey (Norton 1995). Quantification begins by defining a sampling unit. It might comprise either all fish examined, or only those that contain food. Individual fish may be a single unit or combined into a categorical unit (e.g., size, species, or habitat use). Individual prey values that are a ratio (or percentage relative to the total of values across all prey categories) can be calculated either for individual fish and averaged across all fish in a designated unit (i.e., the mean ratio weighted by the number of individual fish), or more commonly as a ratio of the means by first calculating a mean (across all individuals in a unit) for each
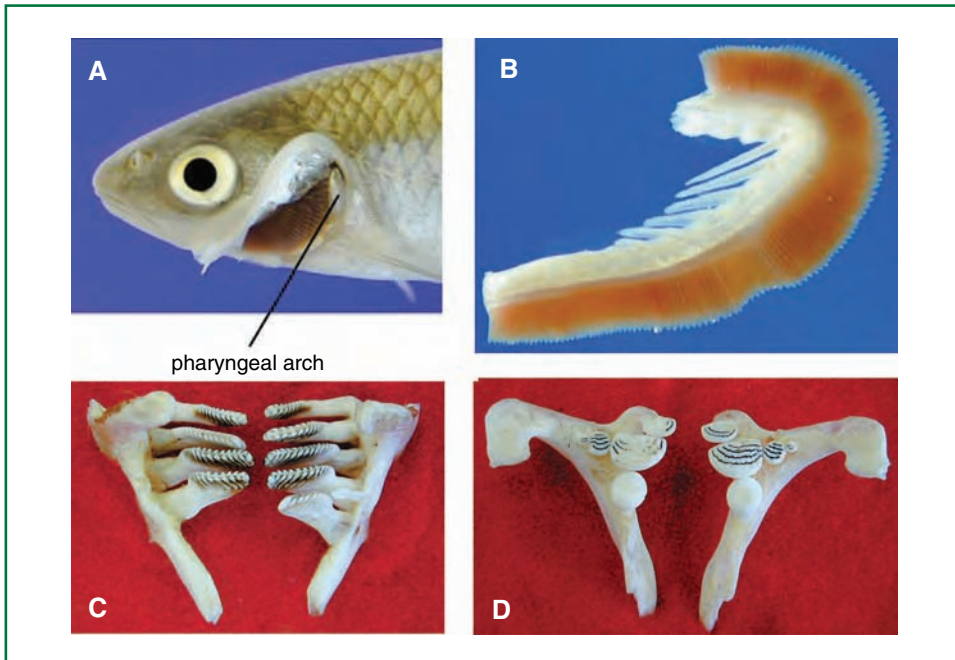
FIGURE 26.2 Location of pharyngeal arch (A) and examples of gill arch (B) showing gill filaments (pink color indicating oxygenated blood) and long spike-like gill rakers that extend into the buccal cavity and pharyngeal teeth (C and D) that are located on the pharyngeal arch from two different fish species having different grinding surfaces. (Photos by Chad Thomas.)

individual prey category and for total prey; then calculate the ratio of each prey category mean to that of the total prey.

1. Volume of a homogeneous mass of small food items can be estimated by flattening the mass to a uniform thickness (e.g., 1 mm) on a Petri dish. Small, yet abundant prey can be diluted from a known volume of gut contents in water and sub-sampled (50 to 100 of each prey type should be counted).
2. Place the dish over graph paper and estimate the area covered for each prey item by counting grid cells, and converting area to volume (Hyslop 1980). A haemocytometer (used for counting blood cells) works well for very small prey items.
3. For larger items impractical to flatten, measure their displacement volume in a calibrated graduated cylinder (Turingan 1994).
4. When the prey item is too small to be weighed accurately or the weight is simply not taken at the time of gut analysis, regression of volume onto mass can be used (see Chapter 29). If items are disarticulated or digested, a characteristic part (best if found once per prey) is counted as one food item and quantified as if a whole item. Alternatively, calculate biovolume (Cummins and Wuycheck 1971; see Chapters 16 and 20).
5. Prey mass of tissues can be measured for wet (blot to remove excess moisture) or dried (desiccation at 105°C to reduce time for bacterial decomposition) items if they are large enough to be handled individually and have been digested only

slightly (Bowen 1983). Total prey mass can be measured by subtraction of empty gut weight from total gut weight before dissection. Because detritus can make up a large part of some diets, stomach contents can be combusted (at 550°C in a muffle furnace) and the ash-free dry mass (AFDM) calculated as dry weight minus ash weight (see Chapter 17). Most prey items can be considered 100% organic matter for these calculations. Adjust the weight of mollusks for shell weight.

B. Basic Method 2: Nondestructive Analysis of Fish Gut Contents

This approach employs nondestructive live release after gut contents are obtained. Fish are anesthetized (see Chapter 22) and assessed for morphology using digital photography (Douglas 1993, McElroy and Douglas 1995) or standard measurements recorded in the field. Stomach contents are obtained with *gastric lavage* (or anal backflushing for fish that have no pyloric sphincter) using various methods including (1) passive gut filling through clear, smooth, nonflexible tubes (plastic or glass) of various diameters with beveled ends to ease their entry onto the stomach, and (2) pumps to force water or other solutions into the gut. These methods require a special apparatus and longer handling time and preparation, so fewer animals can be sampled if field time is limited. Although a simple and effective procedure for most species, gastric lavage can be affected by stomach shape, size of the mouth, and food items of particular fish species (van den Avyle and Roussel 1980, Kamler and Pope 2001, Waters *et al.* 2004), so reliability must be established for each species. In the laboratory, follow the procedures outlined in Basic Method 1 for identifying and quantifying gut contents.

Passive Gut Flushing (Stomach Tubes)

1. After capture, anesthetize fish in an aerated container (see Chapter 22). Complete loss of the fish's equilibrium signals appropriate anesthesia.
2. Prepare a plastic bag (e.g., Whirl-pak®) by placing a label in the bag with information such as stomach number, length, weight, and location where the fish was captured.
3. Wet the end of the tube before inserting it into the fish. For each fish, select the largest diameter tube that easily passes the esophagus without injury to the fish.
4. Using wet hands (to avoid removing protective mucous coating and scales from the fish), one person holds the fish oriented with its head and dorsal side upwards, while another person inserts the wet tube and, via the tube, pours water into the fish's stomach.
5. Once water fills the stomach and is visible in the tube, cover the open tube end, invert the whole fish three times, and then allow water and stomach contents to empty through the tube into a fine mesh aquarium net or container.
6. Repeat the previous step until no additional stomach contents are collected (usually three times).
7. Place the stomach contents in the labeled plastic bag, and store on ice (see alternative preservation method above).
8. Allow the fish to recover in ambient stream water.

Active Gut Pumping

This method employs pulses of water or teleost Ringer's solution (Cavanaugh 1975) to flush the stomach contents (Light *et al.* 1983, Waters *et al.* 2004). Methods used to deliver water pulses include syringes (Culp *et al.* 1988), hand pumps (Seaburg 1957), compression pumps (Foster 1977, Light *et al.* 1983, Hartleb and Moring 1995), and electrical pumps (Crossman and Hamilton 1978). A tube attached to the pump delivers water to the stomach cavity. If using a compression or electrical pump, a means for controlling water flow should be fitted in series with the tubing. This technique can be 100% effective at removing prey items from some species, but much less so (75% or ineffective) for others (Hartleb and Moring 1995). A design for stomach and anal back flushing of small fish (total length 50–85 mm) includes a holding and collection trough, and hand syringes (Culp *et al.* 1988). The design described here is for larger fish and uses a pulsed gastric lavage apparatus (Crossman and Hamilton 1978, Waters *et al.* 2004).

1. Mount a 12-V bilge pump (1,382 L/h) to a Plexiglas frame. Connect a 1.2-m length of polyethylene tubing from the pump to a pistol-grip spray nozzle. To the terminal end of the spray nozzle, attach polyethylene tubing of suitable lengths and inside diameters to closely fit the stomachs of sampled fish. Heat the terminal end of the tubing to round it and facilitate its insertion through the esophagus and into the stomach.
2. Follow steps for fish anesthesia and recovery as described for stomach tubes.
3. Using wet hands, remove a fish and orient it head downward (45° angle) and dorsal side up, while another person inserts the tubing past the esophagus and into the stomach (or past the cloaca into the anus for back-flushing fish that do not have a pyloric sphincter).
4. Holding the tube and the fish's mouth over a fine-mesh aquarium net, trough, or container, turn on the pump. Direct water pulses into the gut and allow backflow to flush out the contents. For large fish, massage the abdomen to help expel water and contents.
5. Continue the previous step until no additional contents are flushed. Transfer the materials to the plastic bag (check the label), seal and place it on ice (or use alternative preservation method).

C. Basic Method 3: Assessing Morphological Structures

Morphology is associated with differential feeding behavior across species, life stages, habitats, and prey types. Some examples are (1) scraping mouth parts for grazing periphyton (e.g., *Campostoma anomalum*, *Acrocheilus alutaceus*); (2) long or finely spaced gill rakers of planktivores (e.g., *Lepomis macrochirus*, *Dorosoma cepedianum*); (3) stout pharyngeal jaws (e.g., *Lepomis gibbosus*) or pharyngeal teeth (e.g., *Moxostoma carinatum*) to crush snail or clam shells; (4) mass of mandibular adductor muscles and jaw opening and closing lever ratios (e.g., *L. gibbosus*); and (5) long coiled guts associated with detritivory and herbivory (e.g., *Notropis nubilis*, *Hybognathus placitus*). Take care to avoid damage to such structures if including them in the study. This method requires a dissecting stereomicroscope, especially for small fish. Structures can be categorized, described, and sketched, or electronic images can be captured using digital photography or x-radiography to locate structures. Digitized data can be further used to quantify overall body shape (e.g., thin-plate splines) and relative change in its components, such as ontogenetic or convergent

trajectories for position of mouth, or depth of head and caudal regions (Svanbäck and Eklöv 2002, Langerhans and DeWitt 2004). For consistency when measuring paired structures, use those on only one side (unless asymmetry also is to be evaluated).

Laboratory Procedures

1. Collect morphological data from whole fishes preserved in the field. Measure external morphological structures prior to dissection for gut contents.
2. Describe or sketch the location, orientation, and morphology of the mouth.
3. Lift the operculum away from the underlying gills and describe or sketch the structure of the gill rakers located on the buccal side of the first gill arch (Figure 26.2A, B).
4. Measure the largest internal dimensions of the mouth by inserting calipers into the fish's mouth in both vertical (gape height) and horizontal (gape width) directions, until a marked resistance is reached; read the measurement directly (or electronically if using digital calipers).
5. Calculate mouth protrusibility as snout length measured with the fish's mouth open minus snout length measured with the mouth closed.
6. Use a stereomicroscope with an ocular micrometer to measure eye lens diameter as a linear distance between the anterior-most and posterior-most part of the lens.
7. On the maxilla and/or the mandible (jaw), count teeth (they might also be present in multiple rows or on the palate or tongue). Measure tooth width and height from the base to highest point on the crown.
8. On the upper and lower limb of the first gill arch (Figure 26.2B), count the number of gill rakers (include rudimentary rakers). Then measure (a) the distance between gill rakers on the medial side of the first gill arch; (b) the length of each raker as the linear distance between the tip of the raker and the center of its point of attachment to the gill arch; and (c) the spacing between the bases of each adjacent pair of rakers.
9. Uncoil the intestine in segments (defined by bends), and measure length of each segment beginning at the esophagus or stomach insertion. Count the number of bends and measure distance between each bend; end the measurement of the last segment at the anus.

D. Advanced Method 1: Morphology of Prey Items

Various aspects of a prey's morphology (e.g., body width, depth, length, and hardness) influence the ability of the fish to consume it. The relationship between measurements of prey morphology and fish size (e.g., body length or gape width) can predict the probability and efficiency of feeding on different prey items.

1. Using a stereomicroscope and ocular micrometer, measure prey maximum width (excluding all legs, spines, wings, etc.) for 100 intact items in the anterior-most segment of the gut (or all items if the gut contains <100 items). For fish prey this is the body depth just anterior to the dorsal fin.
2. For invertebrate prey that are disarticulated, partially digested, or otherwise cannot be directly measured, estimate size by back calculation based on measurements of intact prey items and digestion-resistant structures that can be identified. For partly

digested fish prey, body length and depth can be back calculated based on measurements of abdominal vertebrae or other hard parts from whole fish.
3. Use digital calipers to measure total body length, and a gape micrometer to measure mouth diameter of the predator.

E. Advanced Method 3: Dissection and Removal of Pharyngeal (Throat) Teeth from Fish

This method is used to evaluate pharyngeal (throat) teeth on bones of the last pharyngeal arch, which is embedded in tissue (Fig 26.2A). Number of teeth and their morphology vary among species and are related to trophic habits (Figure 26.2C, D).

1. Push all gill arches forward (or remove carefully for examination) to locate the last pharyngeal arch, which is behind the last gill-bearing arch (Figure 26.2A).
2. Remove the pharyngeal arch by first running a sharp probe (or scalpel on large fish) around the posterior edge of the arch, to sever the muscles at the dorsal and ventral attachment points to the bones of the head. Pharyngeal teeth are primarily on the ventral-anterior side of the arch; therefore, hold the dorsal end of the arch with forceps (use fine No. 5 watchmaker forceps, especially for minnows) and gently pull the arch away. Note how the arch was oriented in the fish and take care to not break off teeth (empty sockets will indicate missing teeth).
3. Carefully remove any tissue remaining around the teeth (often easier after it has dried out) and describe or sketch their morphology.

F. Data Analyses

Diets can be compared among individuals (or categorical units), either as diet breadth and overlap or as diversity and similarity (see Chapter 22), or to compare the diet to prey in the environment (electivity; Strauss 1979, 1982; see also Chapter 24). However, for the latter it is difficult to accurately determine the relative abundances of all potential prey (Ready *et al.* 1985). Although mean values summarize the abundance of prey in a sample, the importance or food value among prey types cannot be compared (e.g., 1 amphipod and 10,000 bacteria). Numerical counts combined with estimates of prey weight and volume, or feeding rate, can be used to calculate indices of relative importance (IRI), bioenergetic relationships, prey biomass or number of prey removed by predators. Relative value of a prey category can be calculated with respect to the total value for all prey categories. The mathematical and statistical properties and biological relevance of the various indices have been debated (Hurlbert 1978, Jumars 1980, Smith 1985). Overall, it is best to choose a relevant, yet simple, index, and to be aware of its limitations when interpreting the analysis (Cortés 1997). Overlap indices are not a true statistic, but they place relationships between species pairs on a relative scale; values >0.75 have been considered as high overlap, whereas values <0.30 as very low (Matthews *et al.* 1982). Overlap (or similarity) in diet and trophic morphology have been used to suggest mechanisms for ecological interaction (e.g., competition, predation) that could affect community structure (Matthews *et al.* 1992).

Data transformations are often needed to satisfy assumptions of parametric tests (i.e., independence, normality, homogeneity of variances, and additivity) for data such as weight distributions for gut contents. For weights reported as a percentage of fish

body weight, the arcsine transform is often useful. Before reporting results based on transformed data, remember to first back-transform means and their standard errors (note that back calculated standard errors are usually asymmetrical around the mean). If transformation does not accomplish your purpose, and especially if the assumptions are grossly violated, use nonparametric techniques and report median values.

Analysis of Gut Contents Data

Basic metrics. Calculate individual data values for a prey category, such as presence of each prey item across all individuals of each fish species (see Table 26.1), or number of items (N), or number of intersections (A) that a prey item covers across a grid of cells of uniform thickness (e.g., counting chamber), or measured volume (V). For each prey category, percent occurrence (%O, Table 26.1), and relative abundance as %N, %A (or V), and %W can be calculated for each sampling unit as:

$$\%O = \frac{O_i}{O_t} \times 100, \tag{26.1}$$

$$\%N = \frac{N_i}{N_t} \times 100, \tag{26.2}$$

$$\%A = \frac{A_i}{A_t} \times 100, \text{ and} \tag{26.3}$$

$$\%W = \frac{W_i}{W_t} \times 100, \tag{26.4}$$

TABLE 26.1 | **Example Data Sheet for Counts of Fish Containing Each Diet Item and Calculated Frequency of Occurrence for Each Fish Species and Diet Item.**

		Diet Items						
Counts:	Total Fish	Fish	Chironomids	Rotifers	Copepods	Algae	Daphnia	Other
Fish Species 1	56	49	25	0	0	5	0	0
Fish Species 2	45	30	5	10	30	0	10	0
Fish Species 3	75	0	0	65	23	52	0	5
Frequency of Occurrence:								
Fish Species 1		0.88	0.45	0.00	0.00	0.09	0.00	0.00
Fish Species 2		0.67	0.11	0.22	0.67	0.00	0.22	0.00
Fish Species 3		0.00	0.00	0.87	0.31	0.69	0.00	0.07

where *i* is the data value composed by each prey category and *t* is the data value for the total of all prey categories in the sampling unit. Alternatively, prey-specific importance can be calculated as above, but for only the individuals that contained the designated prey item in its gut (Welkera and Scarnecchia 2003).

Graphical Methods. Graphical representation of diets can be easier to interpret than tables (Costello 1990, Cortés 1997, Welkera and Scarnecchia 2003, West *et al.* 2003). A simple frequency of occurrence of prey items can be plotted and compared among species (Figure 26.3). Alternatively, greater insight can be obtained by a plot of %O and one or more percent measures of abundance (%N, %A, %V, and %W) to depict prey importance (dominant or rare), as well as predator feeding strategy (specialized or generalized), and the degree of homogeneity of feeding in the predator population (Figure 26.4). Diversity indices also can be used to describe and graph feeding strategies among different stream segments (Welkera and Scarnecchia 2003).

1. Use individual-based data to calculate prey diversity and consumption levels that characterize individual feeding strategies (Bridcut and Giller 1995).
2. Use %O, %N, and %W (or %V) in a two- or three-dimensional plot of data summarized as population-level gut contents.

Indices can also be used to determine if diets are more similar among or within taxa (or size class). Use a *t*-test (see Chapter 35) to compare the average similarities for within-versus between-taxa (or size classes).

1. If a predator manipulation was done, compare the average similarity (Chapter 22) among species in each predator treatment.
2. Use the Levins (1968) index of diet breadth to organize data values (%O, %W, %N) in a matrix of species (or size classes) as rows, and prey categories as columns.
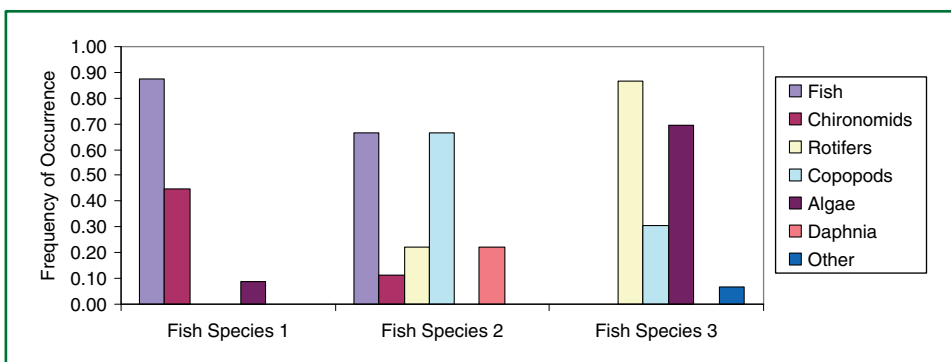
FIGURE 26.3 Frequency distribution of diet items in stomachs of all fish from each of three species in a sample (data from Table 26.1).
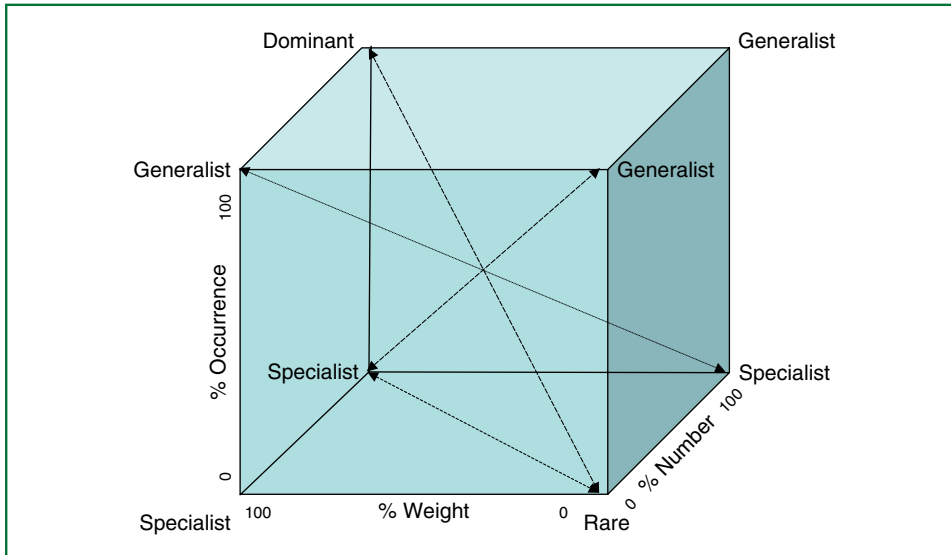
FIGURE 26.4 Three-dimensional graph representing data for gut contents of a fish species, summarized for individual prey categories. Diagonals help to visualize gradients of prey importance (Dominant to Rare) and predator feeding strategies (Generalist to Specialist). (Redrawn from Cortés 1997).

For each fish species (or size class), calculate diet breadth (B) as:

$$B = \frac{1}{\Sigma P_i^2} \qquad (26.5)$$

and standardize it (B_A) to a scale from 0 to 1.0 as:

$$B_A = \frac{B-1}{n-1} \qquad (26.6)$$

where P_i is the proportion of the prey in category i and n is the number of prey categories. B_A can be used as a measure of prey diversity eaten by each fish species (or size class) in a single habitat type or in multiple segments of a stream. If prey were sampled in the environment, use regression analysis to test the null hypothesis of no linear relationship between B_A as the dependent (response, Y) variable, and prey species diversity at collection sites as the independent (predictor, X) variable. This can also be run as analysis of covariance (ANCOVA; Sokal and Rohlf 1995) to test for differences among prey groups (e.g., benthic, water column, terrestrial) or mesohabitats (pools, runs, riffles).

3. Use the coefficient of Morisita modified by Horn (see Chapter 22) to compare diet overlap among pairs of species (or size classes). The coefficient varies from 0 when samples are completely different, to 1 when the samples are identical with respect to proportional prey composition in gut contents.
4. The frequency distribution of the dominant prey items (i.e., prey found in the highest proportion in an individual fish) for each fish species (Figure 26.3), group (e.g., gill raker or pharyngeal tooth morphological type), or experimental unit (e.g., mesohabitat or predator treatment) can also be displayed. Use a G-test (Sokal and Rohlf 1995) to compare the observed frequency distribution of dominant prey for each fish species or group, in each habitat, versus the expected distribution of prey. The expected distribution is based on the frequency distribution of prey items calculated across all fish pooled across all habitats.

Advanced Analyses. More advanced analytical methods employ composite indices to incorporate one or more quantitative methods. One widely used in fish diet studies is the index of relative importance (e.g., IRI; Pinkas *et al.* 1971). In calculating IRI, the percent frequency of occurrence of each prey category is multiplied by the sum of the percent by volume (or weight) and percent by number. The rationale is to cancel biases in the individual component indices (Bigg and Perez 1985), and provide a single measure that more accurately describes dietary importance and facilitates comparative studies, but some consider compound indices as redundant with their singular components (MacDonald and Green 1983). IRI is a composite index used to characterize diets and identify the relative importance of common prey categories (Pinkas *et al.* 1971, Desmond *et al.* 2002). If IRI is expressed as a percentage, this facilitates comparisons among food types (Cortés 1997, Desmond *et al.* 2002). Use three standard dietary measures calculated above (%N, %V, and %O) to compute the IRI:

$$IRI = \%O \times (\%N + \%V) \tag{26.7}$$

1. Calculate the %IRI value for each prey category by dividing the IRI value of the prey item by the total IRI value summed across all prey items (Desmond *et al.* 2002).
2. Characterize general feeding patterns by calculating IRI values for all samples of a particular species pooled across dates and sites.
3. Characterize feeding variability by determining prey IRI values for sample groups ($n \geq 6$) of individual species specific to each combination of sampling date and location, or time of day to determine diel patterns.

You can also use regression to calculate relationships between morphological characteristics of predators and their prey.

1. If you measured predator gape size and prey size, plot the gape size versus largest prey size for each fish taxon of interest.
2. Use ANCOVA to compare these relationships among fish taxa.

Multivariate analysis of variance (MANOVA) can be used to test effects of independent variables on the multiple dependent variables that are the prey categories (Crow 1979).
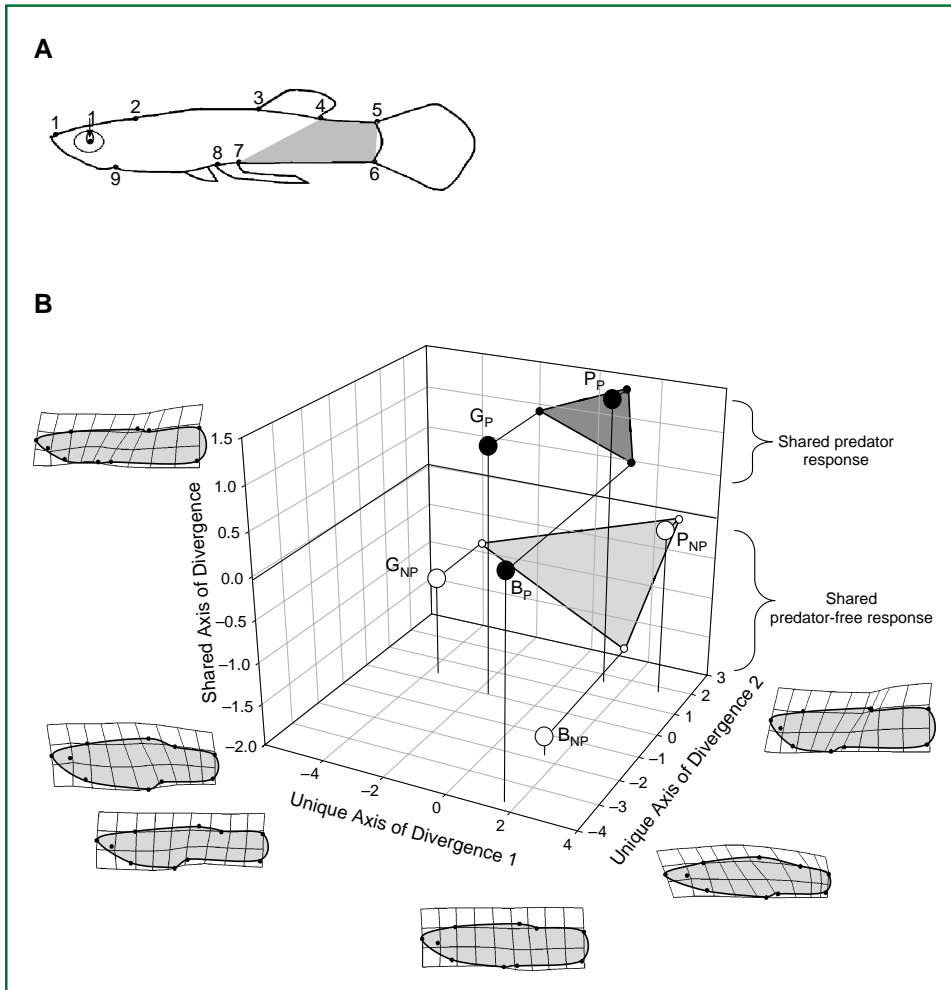
FIGURE 26.5 Location of landmarks (A) digitized to analyze shape change in the mosquitofish, *Gambusia affinis*, and plot of centroids (B) indicating important axes of change in body shape for fish living in habitats either with or without predators (from Langerhans and DeWitt 2004).

1. Test the null hypothesis of no difference in importance values across prey categories (e.g., %A as dependent variables) due to differences among species or stream segments (independent variables).
2. Use MANOVA as a protected F-test by following significant results with post-hoc univariate tests of the independent effects for individual prey categories.
3. Obtain even more information using a canonical analysis of the changes relative to the other response variables (multiple prey categories) that are associated with differential effects of the independent variables (Scheiner 1993). Such analyses include discriminant function analysis (DFA), classification, and multi-way contingency tests.
4. Use ordination software to describe feeding patterns of fish assemblages and explore dynamic feeding relationships over time and space. Nonmetric multidimensional scaling (NMDS; e.g., PC-ORD; McCune and Mefford 1999) and principal

components (PCA) or (detrended) canonical correspondence analysis (DCA, CCA; e.g., CANOCO Ver. 4.5; ter Braak and Smilauer 2002) are used to describe relationships among fish predators and prey in their diets or habitats (Gelwick and Matthews 1992, Desmond *et al.* 2002).

5. Analyze shape to evaluate both shared and unique characteristics of fishes in response to selection gradients such as prey type and habitat within a species as it matures (Svanback and Eklov 2002), or predation across different fish species (Langerhans and DeWitt 2004). Capture lateral images of preserved fish using digital photography (or digital x-radiography for even higher accuracy of landmark locations on the body). Live fish can be restrained in a narrow glass cage (30 mm high × 40 mm long × 5 mm wide) to restrict movement and maintain a constant angle of view by a high-speed video camera. Digitize landmarks (see Figure 26.5A of *Gambusia affinis*; shaded region indicates where natural selection might act to increase fast-start escape speed in the presence of piscivorous fishes). Use geometric morphometrics (e.g., tpsRegr software; Rohlf 2000) to retain information on spatial covariation among landmarks of shape, as compared to traditional approaches using one-dimensional distances. Plot results as a two- or three-dimensional graph (Figure 26.5B) of significant responses for shared divergence (e.g., due to predator presence or absence) by unique divergence (e.g., due to phylogenetic relationships).

IV. QUESTIONS

1. Differences in morphology among species and between populations of the same species can be interpreted as adaptations to different ecological conditions. Describe the differences in morphology you observed in fishes from different mesohabitats (i.e., pools, runs, riffles, vegetated and bare substrate). Are there indications of how they are (or are not) adaptive?

2. How might the presence of a predator affect behavior and growth of two fish species that have different adult body sizes? See Werner and Gilliam (1984) for insight.

3. Discuss why the relationship between gape size and prey size might be stronger within some species or families than others. Why might the strength of this relationship be stronger for the mean, maximum, or overall variation in prey size?

4. If one considers prey encounter rate and vulnerability in evolutionary terms, how would this tend to direct selection processes for predators and their prey in streams?

5. Realistically, even if feeding behavior appears to approach an optimum, there may be overriding functional constraints between the predator and prey depending on the environmental arena within which they interact, leading to suboptimal conditions. Considering the fishes' situation, what constraints to optimization can you identify? How might your expectations now change to more closely match a fish's daily activities?

6. Ontogenetic changes in diet are often associated with differences in habitat, which require alterations in mouth structure. Contrast diet and habitat use for fish in different age or size classes. See Castro and Hernández-Garcia (1995) for insight.

7. Zaret and Rand (1971) showed greatest overlap in diets of fishes in a tropical Panama river occurred during the rainy season, when food is abundant, and

surface runoff yields allochthonous material into the river. Conversely, in the dry season, larger separation of niches occurred. Contrast this with seasonal patterns of diet overlap you would expect for fishes in a north temperate stream system.

8. How would the abundance, endemism, and diet breadth of fishes change their sensitivity to anthropogenic impacts?
9. Predictions of morphology and resource use generally assume that differences in potential resource use (fundamental niche) always leads to differences in actual resource use (realized niche). This may not always be true. Why?
10. How would you test for a functional trade-off due to morphological adaptation for specialization to one resource type, as compared to other resource types? See Robinson *et al.* (1996) for insight.
11. What behavioral mechanisms might influence the magnitude, direction, and consistency of relationships between diet and trophic morphology during early life stages of fish collected from vegetated, rocky, and bare habitats?

V. MATERIALS AND SUPPLIES

Field Equipment

Fish collecting permit and IACUC approval (as required)
4-L plastic jars (one per habitat) with large screw-on lids
Heavy rag or waterproof paper (for labeling collections)
Insulated coolers with ice (for preserving fish)
Pencils or waterproof ink pens (for labeling collections)
Seines: 1.3 m deep × 1.8 m wide and 1.3 m deep × 4.6 m wide (5-mm mesh size, heavy leaded bottom lines)

Alternative Field Equipment for Electrofishing (see also Chapter 22)

Seine: 1.3 m deep × 1.8 m wide (4-mm mesh), for postlarval and juvenile fish
Backpack electroshocker, chest waders, lineman's rubber gloves, long-handled dip nets
Canvas shoulder bags (to accommodate the collecting jars and free hands while collecting)
Concentrated formalin (diluted to 10% in the field for preserving fish)
Polarized sunglasses
Zooplankton net for collecting larval fish

Alternative Field Equipment for Observations

Binoculars for observations from the stream bank
Snorkel and mask or SCUBA gear
Video camera

Laboratory Equipment for Gape Analysis

Blunt and pointed dissecting probes
Dissecting microscope and light source
Fine-tipped scissors
Large flat trays (for sorting and dissecting fish)
Number 5 "watchmaker" straight-tipped forceps
Ocular micrometer (for estimating prey size)

Petri dishes (for stomachs and prey items)
Pipettes (for sorting and counting prey)
Plastic millimeter ruler (150 mm)
Sieve (for rinsing formalin or detritus out of fish collections)
Vernier-type calipers
Wash bottles (for rinsing prey from guts)

Alternative Laboratory Equipment for Shape Analysis

Digital camera
Image analysis software
Digitizing tablet
Access to digital x-ray machine (e.g., veterinary clinic)

VI. REFERENCES

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Stream Food Webs

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I. INTRODUCTION

Stream *food webs* describe the trophic relationships among organisms in streams. An appreciation for stream food webs is essential for integrating the dynamics of organic matter and nutrient processing with community interactions. Food webs differ in structure between stream types, although they all have some common elements (Cummins 1973). Most streams have approximately three or four trophic levels with much connectivity, but disturbed streams typically have simplified food webs (e.g., Townsend *et al.* 1998). Detritus and primary producers, including algae, bryophytes, and vascular macrophytes, occupy the lowest trophic level, but defining higher trophic levels is more complex. Certain groups of macroinvertebrates and some vertebrates can be readily characterized as grazers and detritivores, apparently occupying the primary consumer trophic level. However, both the producers and especially the detritus are intimately associated with heterotrophic microbes and microzoans, which grazers and detritivores also ingest. Thus, these consumers are functioning as both primary and secondary consumers (i.e., somewhere between trophic levels 2 and 3). Predators often have mixed diets including some combination of detritus, diatoms, animal prey, and even other predators, placing them somewhere between trophic levels 3 and 5. In addition, several functional feeding groups of detritivores can be found (shredders, collector-filterers, collector-gatherers; see Chapter 25).

The structure of stream food webs is affected by factors such as biogeography (e.g., Thompson and Townsend 2003), stream order (Vannote *et al.* 1980, Hawkins and Sedell

1981), geomorphology (Pepin and Hauer 2002), substratum characteristics (Lee and Hershey 2000), elevation gradient (Gregory *et al.* 1991), disturbance (Townsend *et al.* 1998, Woodward and Hildrew 2001), temperature (Ward and Stanford 1982), riparian characteristics (Cummins *et al.* 1989, Hicks 1997, Thompson and Townsend 2003), interspecific interactions (Lancaster and Robertson 1995), and nutrients (Peterson *et al.* 1993, Frost *et al.* 2002). Since these factors interact to determine abiotic and biotic conditions in the stream, the food web in any particular stream will reflect all of these factors.

Anthropogenic land uses can strongly influence the supply of nutrients and sediment to streams, which will impact stream morphological features, algal productivity, and fish and invertebrate populations (e.g., Showers *et al.* 1990, Osborne and Kovacic 1993, Lenat and Crawford 1994). Urban and agricultural streams usually have a more open canopy and unconsolidated substrate, especially sand, than undisturbed forested streams in the same geographic region. However, urban streams, which are also strongly influenced by nutrients from lawn fertilizer, runoff, sediments, failed municipal waste (sewage) systems, pet waste, and other pollutants (see Paul and Meyer 2001, Wang 2001), have received less study than either forested or agricultural streams.

Study of a stream food web should begin with identification of trophic levels and linkages. Sampling of organic matter sources and consumer components across habitats is essential. Thompson and Townsend (2003) note that when comparing food webs between streams, it is important to use the same level of taxonomic resolution in all webs. Gut analyses can provide basic information about food sources for consumers (see Chapter 24), although exhaustive sampling and examination of gut contents of hundreds of individuals is required to fully characterize links within the web (Woodward and Hildrew 2001). Much information can be gained in this manner, but several cautionary notes should be considered when examining data from gut analyses. First, for most stream consumers, gut contents will underestimate both the biomass consumed as well as the variety of components, since some diet items may go unrecognized, be macerated beyond recognition, or the predator may ingest only fluids or unidentifiable soft parts. Thus, this approach provides a minimum estimate of the volume and diversity of the diet. Second, for some geographic regions and land use types, consumer diets may change dramatically with seasonal availability of food, ontogeny, or even on a diel basis, requiring a long term and comprehensive study to fully characterize consumer diet items. Third, many consumers ingest material that is difficult to identify. Even a detailed gut analysis effort will result in a diet category labeled "other," or "amorphous detritus," or both. Amorphous detritus includes material of dissolved organic matter (DOM) origin that has become incorporated into the fine particulate organic matter (FPOM) pool, as well as thoroughly processed FPOM that is difficult to characterize as to source. Fourth, depending on the techniques used, gut analyses can overlook important information. For example, bacteria are not evident in guts without special preparation, yet may be extremely important numerically and nutritionally (see Chapter 14). This problem can be overcome with staining (see Walker *et al.* 1988 for DAPI method) or analytical techniques (e.g., Troussellier *et al.* 1993) but specialized preparation and equipment are required. Many fish and some predatory insects swallow prey whole. In these cases, examination of guts provides a good indication of the items consumed, but unless digestion rate is determined and size of prey measured, even these data do not quantify the linkages between a predator and its prey community. Finally, gut analyses provide information about what is ingested, but not all ingested material is necessarily assimilated or assimilated with equal efficiency (e.g., Lamberti *et al.* 1989). Thus, gut analyses may overestimate or

underestimate the energetic importance of many foods. But even with these constraints, gut analyses can be used to construct food web diagrams showing the linkages between food web components. Pimm (1982) refers to such diagrams as "caricatures of nature" but notes that they still contain much information that is of value.

As well as providing information on linkages within a system, food web diagrams can be used to construct hypotheses about population, community, or ecosystem processes and dynamics that can then be tested by manipulating components of the web. For example, Wallace *et al.* (1997) hypothesized that a forested headwater stream food web was energetically dependent on litterfall, then tested the hypothesis by examining whether the food web changed after leaf litter was experimentally excluded (e.g., Wallace *et al.* 1997). It also might be hypothesized that predators control the structure of stream food webs. The hypothesis can be tested by comparing the food webs before and after a predator is introduced (e.g., Townsend 1996, Woodward and Hildrew 2001). Comparisons of food webs from different streams are also very useful in constructing hypotheses regarding differences among streams and/or controls of food web structure within a stream of interest. For example, consider two streams that differed in food web complexity, one with considerable woody debris and another clear of woody debris. One could hypothesize that an observed food web disparity was due to the difference in debris between the streams (e.g., Johnson *et al.* 2003). The importance of woody debris can be tested by either adding or removing woody debris from one stream, and observing changes in food web complexity over time (see Wallace *et al.* 1995). A common exercise used in field courses is to compare the biota of a perturbed stream (e.g., by urban or agricultural runoff) to that of a pristine stream. Food web diagrams can then be constructed from these data, and hypotheses can be generated regarding the mechanisms contributing to any observed differences. Additional information can be gained from experiments designed to explicitly test these hypotheses.

In recent years, the use of stable isotopes in stream ecology studies has become commonplace. Heavy stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) are especially useful for following transfers of carbon and nitrogen from plant and detrital sources to primary and secondary consumers (Peterson and Fry 1987; see Section II.B.2 for definition and discussion of isotope terminology). Technology is developing for using stable isotopes of hydrogen to examine organic matter sources and processing (Doucett *et al.* 2005), and isotopes of other elements also may be used in some circumstances. For example, ^{34}S is especially useful for discriminating marine versus terrestrial nutrient sources for consumers (e.g., MacAvoy *et al.* 2000) but may have utility in freshwater studies in special circumstances, such as in water receiving pulp mill effluent (Wayland and Hobson 2001), or in discriminating among terrestrial detrital sources (McArthur and Moorhead 1996).

In many ecosystems, organic matter sources have different ^{13}C:^{12}C and ^{15}N:^{14}N ratios. Diets of animals can be inferred from the isotopic ratios in animal tissues. The reason for this is simply that 'you are what you eat'. If algae with δ^{13}C$=-30$ per mil (ppt or ‰) and δ^{15}N$=0$ per mil are the sole food of an animal, the isotopic composition of the animal is predicted to be δ^{13}C$=-30$ to -29 and δ^{15}N$=+2.5$ to $+3.5$. Small isotopic shifts (or fractionation) occur between trophic levels due to animal metabolism of carbon and nitrogen compounds. Animal tissues are usually just slightly enriched (an average of $0.3-0.5$ per mil) in ^{13}C relative to their food, but significantly enriched in ^{15}N (an average of 3.4 per mil) relative to their food (see Vander Zanden Rasmussen 1999, Post 2002 and McCutchan *et al.* 2003). The trophic enrichment in ^{15}N is sufficiently predictable that it is often used as an indicator of trophic level (Minagawa and Wada 1984, Vander Zanden and Rasmussen 1999). For example, an animal with a δ^{15}N value of 6 would be

considered to occupy a higher trophic level than one with a $\delta^{15}N$ value of 3.5. Since there is little trophic transfer shift in carbon isotopes, but a relatively large and predictable shift in nitrogen isotopes, the combination of C and N isotopes is frequently used as an aid to determine both pathways of organic matter transfer and trophic structure in ecosystems.

Stream ecosystems sometimes contain natural isotopic distributions that allow an easy differentiation of organic matter sources for different consumers. For example, in a grassland stream, insects might be utilizing either detritus from streamside grasses or epilithic diatoms as food. Many grasses are C_4 plants, which are isotopically enriched in ^{13}C compared to C_3 plants which include riparian trees (see Peterson and Fry 1987). If grass is the primary terrestrial input and has a $\delta^{13}C$ value of -14, and the diatoms have a value of -30, it will be easy to distinguish the food source of detritivores versus grazers. Some species of insects likely will be close to -14 (the detritivores), while others will be close to -30 (grazers), and still others will be in between, reflecting a mixed diet. Top predators are frequently intermediate in $\delta^{13}C$ values between detritus and algae because they feed on both grazers and detritivores. If a prairie stream flows through a gallery forest, detrital sources to the stream may be dominated by litter from C_3 trees, which would be isotopically distinct from grasses. But even in the absence of C_4 grasses, algal and detrital resources often have distinct signatures, especially in anthropogenically impacted streams (e.g., Hicks 1997), and algal $\delta^{13}C$ may vary under different current regimes (Finlay *et al.* 1999).

Although ^{13}C has most often been used to determine food sources for consumers and ^{15}N has been used to delineate trophic levels, ^{15}N can also serve as a tracer of food source under special conditions. In particular, the importance of spawning salmon to stream food webs has been evaluated through the use of ^{15}N derived from salmon carcasses, which are enriched in ^{15}N because salmon are high-trophic level predators in the ocean and some large lakes (e.g., Mathison *et al.* 1988, Kline *et al.* 1990, Schuldt and Hershey 1995, Bilby *et al.* 1996, Fisher-Wold and Hershey 1999, Chaloner *et al.* 2002, Wipfli *et al.* 2003). Stable isotopes of C and S also are often effective tracers for marine-derived nutrients to rivers and lakes (e.g., MacAvoy *et al.* 2000).

Anthropogenic N is often enriched in ^{15}N relative to natural sources. This enriched ^{15}N is reflected in the $\delta^{15}N$ values of stream organisms to the extent that food web components incorporate it. Municipal wastewater or manure from agriculture may have a $\delta^{15}N$ value as high as 15 per mil above nonanthropogenic sources (McClelland and Valiela 1998, Karr *et al.* 2003). However, $\delta^{15}N$ values of fertilizers vary, but are generally depleted in ^{15}N relative to other nitrogen sources because they are produced by chemical reactions that fix N from the atmosphere, which has a $\delta^{15}N$ of 0 per mil (see Peterson and Fry 1987). N-fixing organisms such as legumes are similarly depleted in ^{15}N. Anthropogenic ^{15}N becomes incorporated into producers (McClelland and Valiela 1998) and consumers (Fry 1999), and may therefore serve as a food web tracer if the $\delta^{15}N$ value is significantly different from other N sources (Ulseth and Hershey 2005), similar to what might be seen with ^{15}N tracer addition studies (see following).

Frequently, both detrital and algal organic matter sources have very similar isotopic compositions, making the determination of sources assimilated by consumers from natural isotopic abundance impossible. In such a case, one option is to deliberately introduce an isotopic signal that can be followed throughout the food web. One example would be to add corn leaf detritus ($\delta^{13}C = -13$) to a stream reach that normally receives oak leaves ($\delta^{13}C = -27$). While this might be an interesting experiment, it could be argued that corn is foreign to the system and may not cycle like oak detritus. Another approach

would be to add an inorganic nutrient that is highly enriched or depleted in a heavy isotope. For example, addition of isotopically enriched dissolved ammonium (NH_4^+) will result in ^{15}N enrichment of the algae and aquatic plants that assimilate this nitrogen, followed by enrichment of consumers utilizing those components. Conversely, detritus entering from the riparian zone will not be enriched and detritivores that specialize in using this material would show only a trophic enrichment above the detritus $\delta^{15}N$. Ideally, the tracer enrichment is large relative to the trophic enrichment, but when measuring the tracer enrichment of the consumer, it is important to correct for the expected consumer trophic enrichment (see Kline *et al.* 1990, Tank *et al.* 2000, Rezanka and Hershey 2003). This type of experiment can yield information about nitrogen biogeochemistry and spiralling (e.g., Peterson *et al.* 2001) as well as trophic structure. When applied to a small unpolluted, low-nitrogen stream, the reasonable cost of the isotope and the ease of sampling make this a viable alternative for determining N flow pathways, which are clearly related to trophic structure. Alternatively a ^{13}C-enriched organic compound could be added to a stream to selectively label the bacterial-based food web (Hall 1995). The tracer addition experiment requires more resources and more time than the natural isotope distribution approach. Streams with stable flow for several weeks are ideal but experience has shown that brief freshets have relatively little influence on the results. Larger changes in hydrology can be taken into account by either adjusting the drip rate or more sensibly by calculating the varying daily enrichment and using simple models to evaluate the data (Wollheim *et al.* 1999). Such ^{15}N enrichment experiments have been done in many pristine streams across a range of geographic regions to study N-cycling and biogeochemistry (e.g., Mulholland *et al.* 2000a, Peterson *et al.* 2001, Wollheim *et al.* 2001, Dodds *et al.* 2002) and to trace N-flow through consumers (Hershey *et al.* 1993, Tank *et al.* 2000, Mulholland *et al.* 2000b, Rezanka and Hershey 2003). Recent experiments have focused on denitrification rates and how they vary in urban, agricultural and natural settings (Bolke *et al.* 2004, Mulholland *et al.* 2004).

In this chapter, our objectives are to illustrate three approaches used to study stream food webs — gut contents analyses, stable isotope analyses of N and C, and ^{15}N tracer enrichment experiments — in order to (1) identify the principle sources of organic matter for the stream; (2) assign consumers to trophic levels within the web; and (3) identify specific consumer food sources. Several other approaches also have been used, including functional feeding groups (see Chapter 25), carbon or energy budgets (see Chapter 31), and comparison of food web statistics (see Pimm 1982, Townsend *et al.* 1998, Martinez *et al.* 1999, Woodward and Hildrew 2001). Note that all of these approaches are complementary and in some cases overlapping, but each provides a slightly different type of information. For example, the natural abundance isotope distribution might suggest a link between a particular prey and predator, but the tracer approach might show no linkage. The original idea is challenged but a stomach content study might help decide which observation is most likely correct. In undertaking any stream food web study, the approach or approaches to be used will depend on the questions of interest and the resources available.

II. GENERAL DESIGN

Based on field samples from one or more streams, food web diagrams should be constructed using one or some combination of the methods outlined below or by using functional feeding group analyses (see Chapter 25). The construction of the food webs

may include taxonomic evaluation of samples and gut contents analyses. If organic matter sources with distinct $\delta^{15}N$ or $\delta^{13}C$ values are present in an available study site, this could be exploited for food web studies. Depending on resource availability, a stable isotope tracer experiment could be conducted to measure movement of nitrogen (or carbon, see Hall 1995) through the food web.

A. Site Selection

For the study of stream food webs, it makes the most sense to choose sites for which the maximum prior information is available. Food web studies in such streams may be more meaningful than in streams not previously studied because such studies would complement existing data and contribute to an overall improved understanding of the ecosystem. In addition, knowledge of the taxonomy and life history of the organisms present would facilitate development of an hypothesized food web based on functional feeding groups. Choice of sites, however, must depend on the question being asked. If two or more streams are used, they might be selected to illustrate the range of food web structures available and/or differences in organic matter or inorganic N sources. Suggestions for contrasting pairs of streams or sites include: low order versus high order; low gradient versus high gradient; gallery forest versus open prairie streams; urban versus pristine streams; or upstream versus downstream of a sewage treatment plant or other known or suspected point source of pollution. Natural abundance isotope distributions in different streams vary. The success of an isotope study often depends on finding a site or sites where components of the food web have enough isotopic differences to address the question of interest. A preliminary analysis of a few key samples can help guide the site selection process. A stable isotope tracer addition experiment is most cost effective when performed (see Advanced Method 1 below) on a small stream ($<50\,L/sec$) with low inorganic N concentrations ($<100\,\mu g/L$) because low N flux is crucial to minimizing isotope costs while introducing a strong enough isotopic signal to be useful as a tracer. Adding the tracer as ammonium rather than nitrate may cost less because ammonium concentrations are often much lower and ammonium is preferred by most algae and bacteria in streams.

B. General Procedures

Conventional Food Web Diagrams. A food web diagram should be constructed based on a thorough sampling of stream ecosystem components and gut content analyses (see Chapter 24), possibly combined with an analysis of functional feeding groups (see Chapter 25). The diagram should include about four trophic levels, representing organic matter sources, consumers (positioned according to trophic level), and linkages between source and consumer components. Examples of stream food web diagrams are provided in Cummins and Klug (1979), Power (1990), and Benke and Wallace (1997).

Fractionation of Isotopes and a Hypothetical Food Web. The elements carbon and nitrogen both have heavy and light isotopes that can be used to follow the flow of these elements in ecosystems. The ratios of $^{15}N{:}^{14}N$ and $^{13}C{:}^{12}C$ in components of ecosystems vary in predictable ways as discussed above. The ratios in environmental samples can be measured with great accuracy (a tenth of a part per thousand deviation

from a standard) with mass spectrometers that determine the isotope ratios in the gas from a combusted sample and compare it to a standard. This accuracy is about 10-fold greater than the variation found in most replicate samples from the environment. The standards are carbon from carbonate rock (the Peedee Belemnite formation) and nitrogen in air, for C and N, respectively. Results are usually expressed as del or δ (which refers to deviation) values in parts per thousand (‰, also termed "per mil") difference between sample ratio (R_{sample}) and standard ratio ($R_{standard}$), as follows:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample} - R_{standard})/R_{standard}] \times 1000 \qquad (27.1)$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.

Thus, samples enriched in ${}^{13}C$ or ${}^{15}N$ are isotopically "heavy" and have higher δ values, whereas samples depleted in ${}^{13}C$ or ${}^{15}N$ are isotopically "light" (relatively rich in the lighter isotopes ${}^{12}C$ and ${}^{14}N$) and have lower δ values.

If all environmental samples had identical stable isotope ratios, there would be little information (no signal) in isotope data. However, as carbon and nitrogen cycle in ecosystems the elements undergo fractionation during certain processes (reviewed by Peterson and Fry 1987). This means that during those reactions or processes the light and heavy isotopes move at slightly different rates because they have slightly different mass, with the result that the donor and recipient pools or components end up with different isotope ratios. The common example for carbon is the approximately 20‰ fractionation in CO_2 uptake by trees. The $\delta^{13}C$ value for most trees is about -27 to -30‰. This is less than the -8‰ value for atmospheric CO_2 because ${}^{13}C$ diffuses and reacts more slowly than ${}^{12}C$ during stomatal passage and photosynthesis. This fractionation accounts for the consistent large difference between the carbon isotope ratios in the atmospheric CO_2 pool and the terrestrial biota. In lakes and streams the $\delta^{13}C$ value of dissolved inorganic carbon (DIC) varies considerably because stream and lake waters are not usually in equilibrium with the atmosphere. Thus, in our example below we assign stream algae a $\delta^{13}C$ value of -35‰, which is feasible if the stream is supersaturated with CO_2 derived from the decomposition of terrestrial detritus ($\delta^{13}C = -28$). DIC in such a stream might average -15‰, rather than the atmospheric value of -8‰, reflecting that it is derived from both atmospheric and respiratory CO_2 sources. Several factors can affect algal fractionation of C (e.g., see Finlay *et al.* 1999), but if, as an example, algal fractionation of C is 20 per mil, the ${}^{13}C$ signal should be $-15 - 20 = -35$‰. In contrast, nitrogen fixation by microbes and plants often exhibits little fractionation and it is not uncommon for plants to have $\delta^{15}N$ values close to the 0‰ atmospheric value. However, microbial processes such as nitrification and denitrification, and animal metabolism, fractionate nitrogen isotopes sufficiently such that all ecosystems contain components with significant (>1‰) variation in N isotope ratios.

An example of a hypothetical stream food web analyzed using stable isotopes is shown in Figure 27.1. Initial study of this stream has shown that it receives large amounts of leaf detritus, but also has sufficient light input to support a benthic diatom community. Samples of detritus, epilithic algae (diatoms), insects, and fish have been collected and analyzed for C and N isotope ratios. As expected, the tree leaf detritus had a $\delta^{13}C$ value of -28 and a $\delta^{15}N$ value of 0. Absolutely clean samples of diatom cells are very difficult to collect in the field or extract in the lab (see Hamilton *et al.* 2005) as the
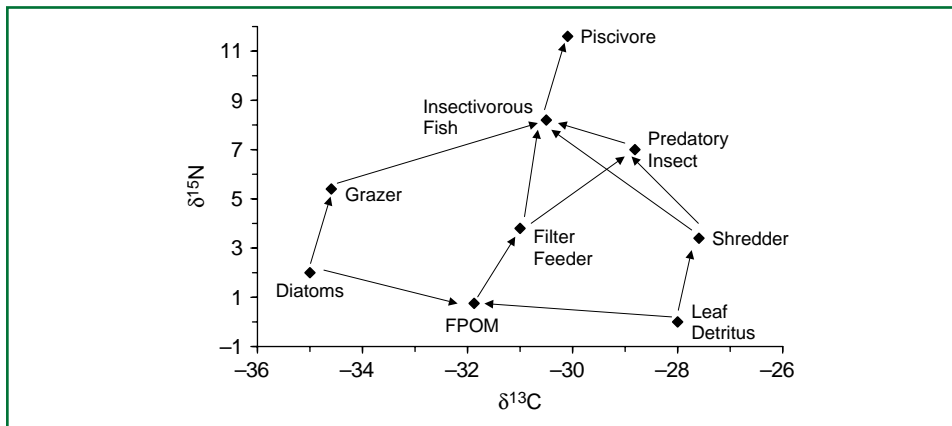
FIGURE 27.1 Sample plot of hypothetical values for $\delta^{13}C$ versus $\delta^{15}N$ for various components of a typical stream food web. Arrows indicate hypothesized trophic transfers based on a fractionation of approximately 3.4 per mil for N and 0.4 per mil for C for each trophic level.

cells grow in an epilithic or epibenthic matrix of microbial slime and detritus, but for illustrative purposes we assign them a $\delta^{13}C$ value of -35 and $\delta^{15}N$ value of $+2‰$. Assuming a literature based trophic transfer shift of $+0.4$ per mil for C and $+3.4$ per mil for N (Post 2002), the predicted values for insects with contrasting feeding modes and predators is shown in Figure 27.1. Note the wide separation in $\delta^{13}C$ values for consumers feeding upon diatoms versus detritus, clearly indicating their organic matter sources. Also note that predators have higher $\delta^{15}N$ values than their prey. FPOM is derived from both algal and detrital components and has a $\delta^{13}C$ values that is intermediate between algae and detritus (Figure 27.1). The system has about four trophic levels. While this system is oversimplified for illustrative purposes, it is not very different from what we see in many stream ecosystems (Fry 1991). In real ecosystems, there are likely to be more than two organic matter sources and many consumers are likely to have more generalized (mixed) diets, leading to less clear isotopic separation and more ambiguity in interpretation of organic matter transfer through the food web. Such limitations of natural abundance isotope studies emphasize the importance of having additional information from gut contents, feeding studies, morphological analyses, and/or tracer experiments.

When a consumer has an intermediate isotope value between two sources, a simple two-source mixing model can be constructed to evaluate the relative contribution of each source to consumer diets (e.g., Kline *et al.* 1990). An equation for a simple two-source mixing model for $\delta^{15}N$ of a consumer feeding on a mixture of algae and detritus, corrected for a trophic shift of 3.4, is as follows:

$$\delta^{15}N_{consumer} = [s \cdot \delta^{15}N_{algae} + (1-s) \times \delta^{15}N_{detritus}] + 3.4 \qquad (27.2)$$

where s = proportion of the diet derived from algae and (1-s) is the proportion derived from detritus. This can be rearranged to calculate the proportion of the diet derived from algae:

$$s = (\delta^{15}N_{consumer} - \delta^{15}N_{detritus} - 3.4)/(\delta^{15}N_{algae} - \delta^{15}N_{detritus}) \qquad (27.3)$$

Oftentimes, there are multiple likely basal sources for food web components such that a two-source mixing model is not suitable. Phillips and Koch (2002) have developed a mixing model that evaluates contributions of 3 sources when signals for two isotopes are available, which also accounts for differences in proportional contribution of each isotope. Their model can also be generalized to $n+1$ sources, when δ-values for n isotopes are available. In addition, for cases with $> n+1$ sources, Phillips and Gregg (2003) have developed a method (IsoSource) which gives the range of possible contributions from the different source materials for which isotopic data are available. Regardless of the apparent power of these mixing models, variation in diet composition over time, variation in the relative digestibility of different organic C and N sources, and natural variability in trophic enrichment for ^{15}N can lead to errors in interpretation if ancillary information from alternative approaches is not take into account.

Influence of Urbanization on Food Webs. Urban development can have significant impacts on the trophic dynamics of streams, and result in food webs that are quite different than those in pristine streams due to differences in species tolerances to various disturbances (e.g., Lenat 1988, 1993). One of the potential impacts of urbanization on stream food webs is the introduction of anthropogenically derived N and C (McClelland and Valiela 1998, Ulseth and Hershey 2005). These elements may come from point source inputs (e.g., wastewater treatment and industrial effluents) and non-point source inputs (e.g., storm water runoff, sewage leaks, golf course and lawn fertilizer). Since anthropogenic sources of N and C often have stable isotopic signatures that are unique relative to more natural sources, longitudinal changes in the isotopic signature of stream food web components can provide information about inputs of anthropogenic N and C into the food web. Although nonpoint source inputs may be isotopically unique, they are spatially and temporally diffuse and thus will produce detectable isotopic changes in stream food web components only if they are large in magnitude. However, point source inputs are spatially constrained. As a result, stream food web components that are incorporating isotopically unique anthropogenic N and C from a point source input will show a distinctive isotopic signal below the source, compared to above it (Ulseth and Hershey 2005; Figures 27.2 and 27.3). Furthermore, since the isotopic signature of a component represents a time integrated signature of all of its N and C sources (O'Reilly *et al.* 2002), longitudinal changes in component signatures can reveal even temporally variable point source inputs.
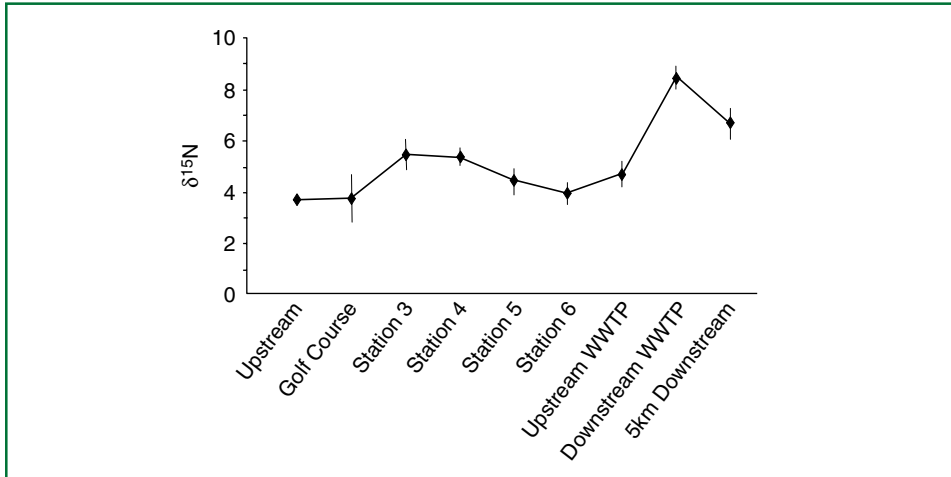
FIGURE 27.2 Transect of $\delta^{15}N$ signature of seston (mean $\pm$ SE) in North Buffalo Creek as it passes through the City of Greensboro, NC. Note the ^{15}N enrichment below the Golf Course site, return to near baseline, followed by a greater increase at the Downstream WWTP site (below the North Buffalo Creek Waste Water Treatment Plant). (From Ulseth and Hershey 2005).

III. SPECIFIC METHODS

A. Basic Method 1: Construction of Food Web Diagrams Based on Consumer and Resource Samples and Gut Analyses

This exercise is a cursory study of organic matter sources using several specimens of a few dominant consumers to be completed by researchers working in groups for 6–8 hr. A detailed, quantitative analysis would take one person several months to complete.

1. Collect samples of food resources and consumers. In representative stream habitats, sample primary producer groups (e.g., algae, vascular macrophytes, bryophytes) in each habitat (see Chapters 16–18), detrital CPOM and FPOM components (see Chapters 12 and 13), invertebrate macroconsumers (see Chapters 20 and 25), and fish (see Chapter 22).
2. Sort invertebrate samples into functional feeding groups (see Chapter 25 or Merritt and Cummins 1996).
3. Determine gut contents of several individuals from each common species or taxonomic group (see also Chapter 24). Choose those groups that are most common if samples are very diverse.
   a. To perform gut analyses, first dissect guts from animals. The ease of this dissection varies among invertebrates, but with patience, can be accomplished for even small invertebrates. For many invertebrates, especially insects, the entire gut can often be extracted by holding the abdomen firmly (but not squeezing) with fine-tipped forceps, then pulling the head away from the body using another pair of fine-tipped forceps. Often the gut will come out attached to the head. When this does not work, the gut can sometimes be pulled out of the abdomen once the head is removed, especially if an incision is made
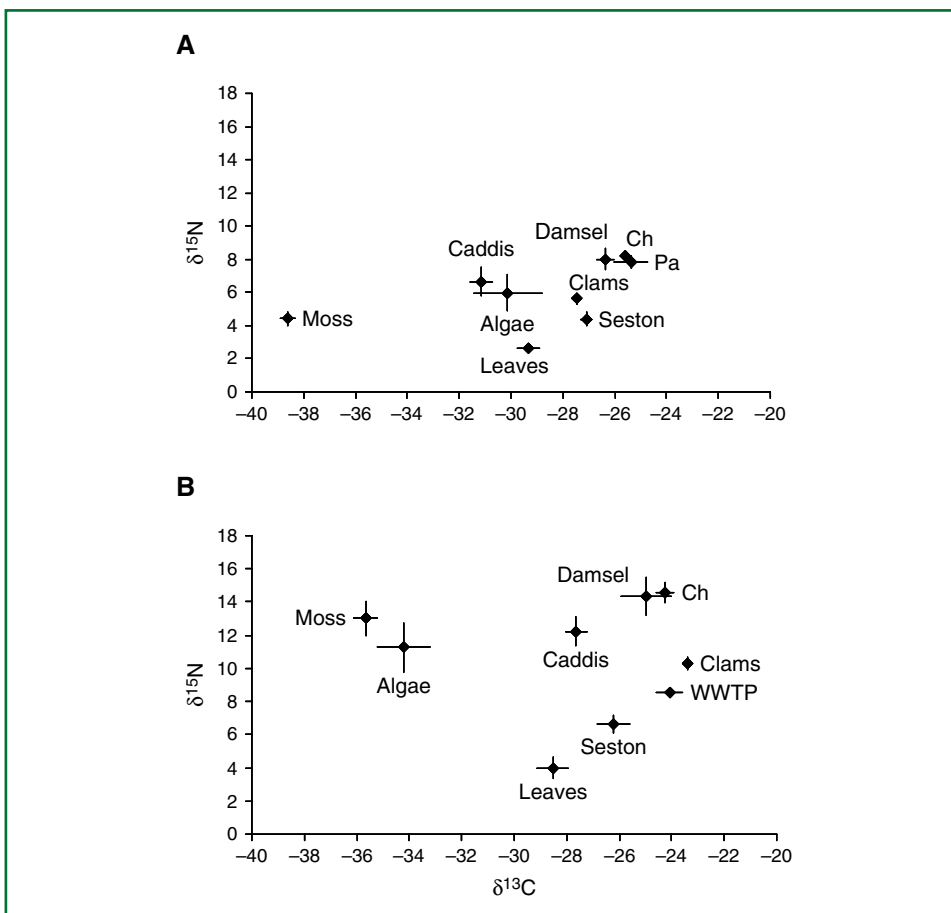
FIGURE 27.3 Isotope cross-plots of dominant food web components in North Buffalo Creek, NC, at (A) a site not impacted by point source inputs and (B) a site downstream of a waste water treatment plant. The comparison between the two cross-plots illustrates that most components show shifts in both $\delta^{15}N$ and $\delta^{13}C$ indicating incorporation of sewage-derived N and C into the food web. Caddis = Hydropsychid caddisflies, Damsel = damselflies, clams = Asian clams, Ch and Pa = *Cambarus* and *Procambarus* crayfish. (From Ulseth and Hershey 2005).

longitudinally along the ventral side of the abdomen. Use a fine, sharp dissecting pin for the incision. Care should be taken to remove the gut intact whenever possible. For taxa where the foregut is distinct from the midgut, it would be preferable to use only the foregut.

b.   Once the intact gut has been dissected, place it on a clean microscope slide in a drop of mounting media. Tease gut contents from the gut or foregut with a fine dissecting pin or fine forceps, and disperse in the mounting media. Place a cover slip over the preparation, press gently to spread the gut material, and then dry the slide on a slide warmer.

c.   Examine the gut contents and identify components as accurately as possible. This is not unlike solving a puzzle, since gut contents are often fragmented. Knowledge of the stream flora and fauna, and prior microscopic examination

of FPOM, is very helpful. Diatoms and filamentous algae are often in good condition, and may be identified to genus or even species using appropriate sources (see Chapter 16). Detrital material can usually be characterized as vascular plant detritus, animal detritus, amorphous detritus (e.g., FPOM of DOM origin), or other categories that can be recognized. It may be desirable to quantify the relative importance of various types of detrital and algal foods by estimating the area covered by each in a predetermined and constant number of randomly selected fields on a microscope slide, or by measuring algal biovolume (see Chapter 16). Invertebrate diet items may or may not be intact depending on the consumer, but often fragments including such things as head capsules, sclerites, or claws may be identifiable if the stream fauna is well known. This often involves matching fragments against comparable characters of taxa that have been previously keyed from the habitat. Record your findings on a data sheet, such as Table 27.1.

4. Sort and identify fish (see Chapter 22). Remove stomachs and conduct stomach analyses on these specimens, similar to invertebrate gut analyses. For predatory fish, dietary items are often intact or nearly so, and individuals can be keyed using the appropriate references (see Chapter 20; Merritt and Cummins 1996, Thorp and

TABLE 27.1	Example Data Sheet for Gut Contents Analyses. For each specimen, identify the specimen and itemize each of the items found in its gut (or stomach for fish). Food items should be identified to the extent possible, and each type should be enumerated. An example is given. For items that cannot be enumerated, categorize them as abundant, common, or rare (establish criteria for these categories and use them consistently — for example, rare <5%, common 5–20%, abundant >20%).

Stream or stream reach:
Habitat:
Collection date:
Investigator:
Notes:

Specimen	Gut contents for each specimen.					
	Animal prey	Algae	Plant detritus	Amorphous detritus	Other	Comments
1. *Rhyacophila*	3 *Prosimulium* hypostomas 1 *Cricotopus* head capsule 2 *Tanytarsus* head capsules 1 *Simulium* head 2 *Baetis*? claws	3 *Cladophora* filaments	rare	common	–	Some gut material lost during dissection.
2. etc.						

Covich 2001). Herbivorous and detritivorous fish are more challenging. For preliminary work, disperse stomach contents in a small amount of water in a petri dish. Pick out large items and identify using a dissecting scope, or mount on slides as necessary. Smaller amorphous material can be subsampled with a pasteur pipette and mounted on slides. You may want to prepare several slides per animal.

5. Based on collections of organic matter and gut analyses, categorize each animal taxon according to the specific food items consumed, and according to the appropriate trophic category.

6. Construct a food web diagram from your data. Organize the data vertically with organic matter sources at the bottom, predators at the top, and primary consumers in between. Connect consumers with their food using arrows indicating the direction of energy flow. For organic matter sources or consumer groups at the same trophic level, organize these laterally in the web at the same respective vertical level.

B. Basic Method 2: Analysis of Food Webs Using Stable Isotopes

1. Prepare samples of components collected in Basic Method 1 for isotope analyses, and send to a university or commercial laboratory for analysis of $\delta^{13}C$ and $\delta^{15}N$ (unless your own institution has the capability to analyze stable isotopes).

2. Collect samples for stable isotope analyses consisting of a few to several mg dry mass of leaves, mosses, algae, or animal tissues. Animals should be held in clean filtered stream water in a cool place for at least several hours, or overnight, to help clear their guts (the contents of which might bias the isotopic signal). Following gut clearance, crustaceans, snails, or bivalves should be removed from carbonate shells because carbonate will have a very enriched $\delta^{13}C$ value compared to animal body tissue. Care should be taken to separate individuals to species level if possible since even closely related species sometimes have quite different diets. Frequently, it is both necessary and desirable to pool several to many individuals of a species for a single analysis. While it is interesting to know the individual variability, the sample quantity and cost considerations usually require pooling. Furthermore, pooling many individuals may give a better estimate of the mean value for the population from a site. For larger organisms, it is possible to analyze specific tissues, but in this exercise we will use either whole body analysis or muscle tissue (for fish or crayfish).

3. Place clean samples in glass scintillation vials or microfuge tubes and dry in an oven at 60°C, or use a freeze drier, if available. Dried samples can be held indefinitely in a dessicator. Alternatively, samples can be frozen for later dissection and drying. Ethanol-preserved or formalin-preserved samples can be used, but the preservation will result in some alteration of both C and N δ-values (Hobson *et al.* 1997, Arrington and Winemiller 2001, Heidrun and Grey 2003).

4. Dried samples can be sent to any of several commercial or university laboratories specializing in mass spectrometry. Several university labs offer combined $\delta^{13}C$ and $\delta^{15}N$ analyses and C/N ratio in the range of $9 to $15 per sample. It is worth shopping around on the Internet and/or making some inquiries to find an affordable price. Prices sometimes vary with the amount of preparation done by the investigator, so you should check the sample preparation expectations for the lab where you plan to send samples. Commonly, dried samples should be ground to a powder, weighed, and sealed in 4×6 mm tin capsules, or placed in clean glass vials or microfuge tubes. Homogenizing samples very thoroughly is important for

accurate determinations because variation among subsamples can be surprisingly high (several parts per mil) if samples are not finely ground and thoroughly mixed. If you plan to do serious or continuing work, send a known blind sample as a standard with each shipment to increase your confidence in the resulting data. Because isotopic analyses are expensive, it is prudent to analyze a few preliminary samples of either organic matter sources or various types of consumers to discover whether or not your system contains naturally contrasting $\delta^{13}C$ and $\delta^{15}N$ values. If the system turns out to have large signals and is well poised isotopically for your question, then analyze additional samples.

5. Your data will arrive on a data sheet or spreadsheet from the isotope laboratory, and will look similar to Table 27.2. First plot all the data for $\delta^{13}C$ and $\delta^{15}N$ separately to look for the range of values and the pattern of grouping of $\delta^{13}C$ and $\delta^{15}N$ values (see Fry 1991 for examples). A rule of thumb is that values that are different by 1‰ or less should not be considered different unless you have enough true field replicate samples (not subsamples from the same vial) to compute an accurate variance. While the precision of laboratory analyses may be better than 0.1‰, the variability in most ecosystem components and in sampling/processing is almost always larger (Fry 1991; see also Figure 27.2).

6. Next construct an isotope cross-plot of $\delta^{13}C$ versus $\delta^{15}N$. Draw in your hypothesized food web pathways based on gut analyses. Construct a similar plot with the food web pathways drawn as suggested by the isotope data and expected trophic fractionation (similar to Figure 27.1). Do the gut contents and isotope approaches agree? Do the isotopic distributions support your morphological observations and functional feeding group classifications? You can combine the two approaches to check for consistency. If your stomach content data show that prey items 1, 2, and 3 are present in proportions by weight (or C content) of 0.2, 0.5, and 0.3, respectively, then you can calculate a predicted consumer $\delta^{13}C$ value as follows:

$$\delta^{13}C_{consumer} = (0.2 \times \delta^{13}C_{prey\ 1}) + (0.5 \times \delta^{13}C_{prey\ 2}) + (0.3 \times \delta^{13}C_{prey\ 3}) \qquad (27.4)$$

You might add 0.2 per mil for trophic enrichment but this is within the noise level in this calculation. How does the calculated value compare to the measured value for the consumer? You can repeat the calculation for nitrogen but be sure to add the 3.4 per mil trophic enrichment factor.

TABLE 27.2	Sample Stable Isotope Data from a University Laboratory.			
Code	**Sample ID**	**$\delta^{13}C$**	**$\delta^{15}N$**	**Comments**
1	Lab Creek oak detritus	−28.0	0.0	
2	Lab Creek "diatoms"	−35.0	2.0	small
3	Lab Creek black flies	−26.8	4.0	
4	Lab Creek snail	−18.1	5.4	carbonate contamination?
5	Lab Creek minnow	−30.2	7.2	

7. Usually the answers to these questions do not come easily because natural food webs are complex, with temporal and spatial variability, as well as species changes that confound any simple interpretation. Equally important is that the different approaches to food web analysis provide different kinds of information. Gut contents indicate what a consumer has eaten recently, but isotopes help identify the original source of the organic matter, how many trophic links are involved in the transfer from source to ultimate consumer, and integrate a longer-term feeding history of the consumer. Except in unusually simple systems, isotopes tell little about the species composition of diets. It is best to use either a combination of these complementary approaches or to use the approach that comes closest to answering your specific question.

C. Basic Method 3: Identification of Point or Non-point Source Inputs of Anthropogenic N and C

This exercise is designed to identify non-point and point source inputs of anthropogenic N or C by detecting the shift in the isotopic signature of stream food web components that results from the incorporation of an N or C source having a unique isotopic signature. Upstream and downstream of an urban area that lacks any point source discharges may provide contrasting isotope signatures due to non-point source influences, and upstream and downstream of a sewage treatment plant or industrial effluent would likely provide contrasting isotope signatures due to point source inputs.

1. Identify the stream reach or reaches to be sampled. Since the purpose of this exercise is to screen for potential point source or concentrated nonpoint inputs of anthropogenic N and C, it is useful to sample a stream reach that receives large potential point or nonpoint source inputs relative to its discharge. To obtain sufficient resolution in identifying changes in N and C isotope signatures, food web components should be collected at numerous sites along the chosen stretch of stream or streams. The exact number of sites chosen will depend on the resources available for sample collection and isotope analysis. Preliminary analysis of a few taxa along the reach will help identify the optimum locations for more intensive study. Choose sites that are expected to be relatively unimpacted (e.g., above a municipality) and sites that are expected to receive some isotopically distinct inputs (see Figure 27.2).

2. Identify the food web components to use as isotopic tracers. Because comparisons will need to be made between the signature of each component at each of the different stream sites, it is important that the same components are collected from all (or nearly all) of the sites in the longitudinal transect. It is often necessary to limit sampling to one or a few easily collected food web components (e.g., periphyton, seston) and relatively ubiquitous and pollution tolerant consumers. Some examples might be net-spinning caddisflies, crayfish, Asian clams, or a common stream fish. If resources are available and the stream is large enough so that sampling will not deplete organisms or overly disrupt the ecosystem, plan to sample 3 or more replicates of each component.

3. Collect the components from each of the sites along the transect.

4. Process organisms for stable isotopes as described in Basic Method 2.

5. Look for longitudinal trends in the data. Often the best way to identify point source inputs is to plot the isotopic signatures of the consumers by the stream distance

from beginning of the transect. Abrupt changes in isotope δ values are indicative of a change in the N or C source of the food web which may be attributable to a point source (e.g., Figure 27.2). Are any point source inputs evident on your graph? Do you see gradual changes that may be consistent with non-point source inputs?

6. Characterize the food web below a known point source input of N or C. Once a point source input of anthropogenic N or C has been identified as discussed above, or if one is already known (e.g., a sewage treatment plant), a more detailed investigation of the food web can be performed above and below the source of the input to determine which organisms are utilizing the anthropogenic N and/or C (e.g., Figure 27.3). Examine your data using isotope cross plots. Look for shifts in basal food web sources, and in the isotope signature of dominant consumers. If you collected multiple samples from each site, food web components can be compared above and below a point source effluent using t-tests. Does the point source alter the structure of the food web? In what way or ways? Did you learn anything from this type of analysis that you might not have learned from gut analyses?

D. Advanced Method 1: Experimental Manipulation of ^{15}N in a Small Stream

An experimental approach to studying use of autochthonous sources in the food web is to label the inorganic nitrogen in a stream by dripping ^{15}N-enriched NH_4^+ or NO_3^- directly into the stream (i.e., introduce a highly enriched isotopic tracer). This approach labels the components of the ecosystems most heavily dependent on autochthonous production because stream algae and aquatic plants rapidly assimilate NH_4^+ and NO_3^-. Enriched compounds are available commercially from many chemical suppliers and the purchase cost of adding a readily detectable isotopic signal to a small stream can be as low as $50 to $500, depending on mean discharge and background nutrient concentrations. Since NH_4^+ concentration is likely to be much lower than NO_3^-, it is probably considerably more economical to use NH_4^+. The experiment involves the following steps:

1. Calculate the feasibility of tracer addition by determining the amount of ^{15}N required to elevate the δ-value of in-stream N by 100 per mil or more for several days or weeks (e.g., Table 27.3). Check calculations carefully. An unanticipated high level of isotopic enrichment of food web components could result in contamination of other samples run on the same machine, whereas underestimating the target level of enrichment may result in a signal too small to resolve from background variation.

2. Determine the optimum study reach and conduct baseline sampling prior to enrichment. Plan to have an upstream control reach and a downstream reach of from 100 m in a very small (0.01 m³/s) stream to 1 km in a slightly larger stream (0.1 m³/s). The baseline sampling is the same as in the natural abundance exercise above but should be conducted along upstream–downstream transects to uncover any large natural isotopic gradients that might be due to spatial variation in nitrogen inputs or in-stream processes.

3. Test the ^{15}N-delivery apparatus for reliability, as continuous and accurate delivery is important. During a season of active algal growth and low flood frequency, deliver the tracer to the stream by means of a continuous drip at a turbulent site for a period of 4–6 weeks. Introduction of ^{15}N can be via Marriott bottle (see Chapter 8, Figure 8.1) or peristaltic pump (Figure 27.4; see also Chapter 10). The reason for a continuous addition is that it takes a period of several days for primary producers and up to several weeks for consumers to become well labeled. A pulse addition

TABLE 27.3 Sample Calculation Steps for a Stream with Discharge of 100 L/s (= 0.1 m³/s) and NH_4^+-N Concentration of 5 μg/L.

1. Calculate daily flux of nitrogen as follows:
   daily flux $= 0.1 \, m^3/s \times 60 \, s/min \times 60 \, min/h \times 24 \, h/d \times 5 \, mg/m^3 = 43200 \, mg/d$ or 43.2 g/d.

2. Determine the daily flux of ^{15}N-NH_4^+. The daily flux of ^{15}N due to the natural levels of approximately 1 g of ^{15}N for each 273 g of N is found by dividing the NH_4^+ flux by 273. Thus, the flux of ^{15}N is 43.2 g/d divided by 273, or about 0.158 g/d of ^{15}N.

3. To obtain a target 100‰ enrichment of ^{15}N (not ^{14}N or total N!), we want the ^{15}N flux to be increased from 0.158 g/d to $0.158[(100/1000)+1] = 1.1 \times 0.158 = 0.174 \, g/d$. The difference of 0.174 − 0.158 or 0.016 g is the amount of ^{15}N isotope needed for each day of the experiment. If the experiment is to continue for three weeks, $21 \, d \times 0.016 \, g/d = 0.336 \, g$ of ^{15}N will be needed.

4. Commercial compounds enriched in isotopes are usually sold by the gram of compound, not element. For example, for purchasing ammonium chloride, the quoted price is for grams ammonium chloride, not grams ^{15}N. Also note that commercial firms offer different enrichment levels from 5 to 99%: 5% enrichment means that 5% of the N atoms are ^{15}N as compared to 1/273 or 0.3663% occurring naturally. The lower % enrichments are satisfactory for these experiments and may or may not be less expensive per gram ^{15}N.

5. If we choose to buy 5% ^{15}N-ammonium chloride, you will need to calculate how much ^{15}N it contains per gram in order to purchase the correct amount of isotope. A mole of NH_4Cl weighs 53.45 g, approximately 14 g of which is N. Therefore, 1 g of NH_4Cl contains $(1 \, g)(14 \, g/mole \, ^{14}N)/(53.45 \, g/mole \, NH_4Cl) = .262 \, g$ N. If a product is enriched 5% with ^{15}N, then 5% of its N atoms will be ^{15}N rather than ^{14}N, or $(.05)(.262) = 0.0131 \, g$ ^{15}N per gram NH_4Cl. To purchase the needed 0.336 g of ^{15}N, we would need to purchase 0.336 g ^{15}N/0.0131 g ^{15}N per g $NH_4Cl = 25.7 \, g \, NH_4Cl$ that is 5% enriched. If 5% enriched NH_4Cl costs about $5 per gram, the cost of the isotope tracer would be about $125. At higher discharge or higher NH_4^+ concentration in the water, more would be needed. For example, if stream discharge were 0.3 m³/s, rather than 0.1, and NH_4^+ concentration were 10 mg/L rather than 5, you would need six times more isotope to stay at the 100‰ target enrichment level, and the cost would be $750. These calculations would need to be adjusted for a different %^{15}N ammonium chloride or a different target enrichment level. Choose your stream carefully because tracer experiments even in small streams with high ammonium (or high nitrate if you choose that form) concentrations can be prohibitively expensive.

might result in too small a signal at the upper trophic levels. A continuous addition for several weeks allows the investigator to track the strength of the signal via sequential sampling to determine the rate of N uptake and the asymptotic δ-value of many ecosystem components, as well as the rate of N loss after the addition ends.

4. Once the ^{15}N addition has started, sample algae, insects, small fish and detritus such as leaves and fine benthic organic matter at a series of stations downstream of the dripper. Also sample one or more stations in the upstream control reach. These reference samples will show if there are temporal changes in background isotope values during your experiment. Locate the first experimental station very close to the dripper but where you expect the isotope to be well mixed across the stream channel. The best location can be determined beforehand using a dye such as rhodamine or fluorescein to observe mixing in a stream channel. Locate the last station well downstream of the point where you think most of the tracer will have been taken up. Sample three to eight stations in between but more closely spaced
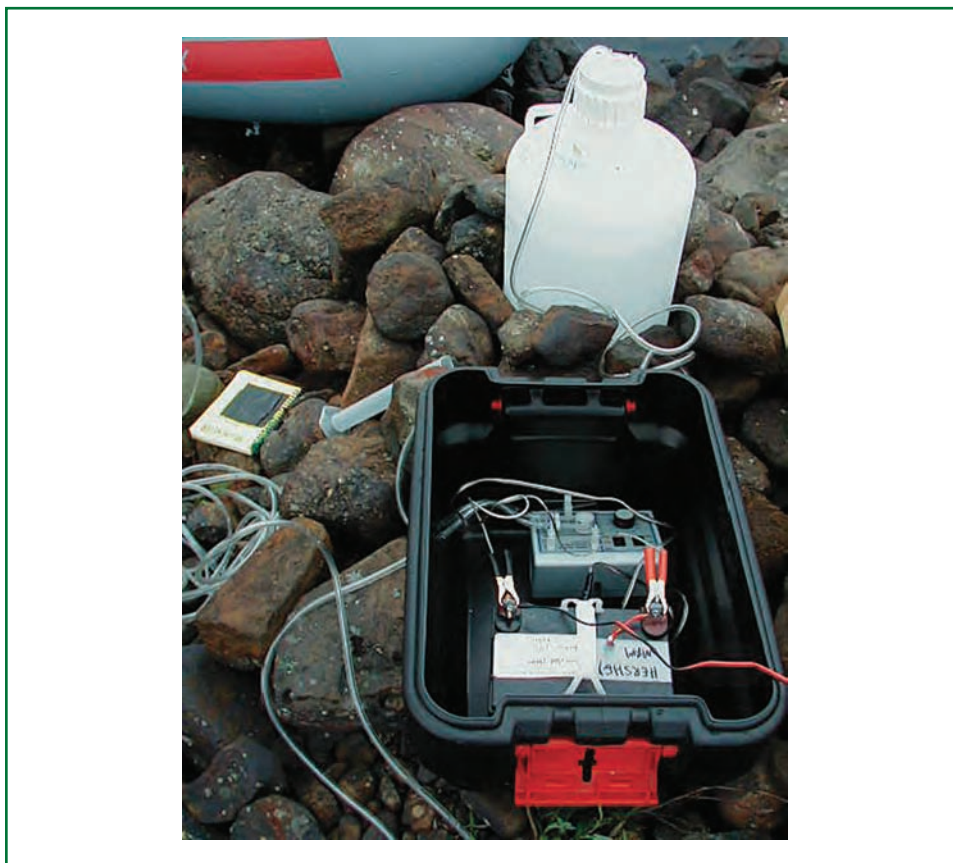
FIGURE 27.4 Battery-powered pump system for delivering $^{15}N\text{-}NH_4^+$ to a stream. In this system, the battery is powered by a solar panel (not shown), but where light is limiting, the battery can be changed as needed.

near the dripper. A progressive station distribution such as 10, 20, 40, 80, and 160 m works well. Care should be taken to not deplete the study reach of organisms from "oversampling."

Uptake lengths for NH_4^+ in typical unpolluted streams at low discharge range from a few meters in the smallest first-order stream to a kilometer or more in fourth-order streams (Peterson *et al.* 2001). Uptake lengths in a stream reach vary depending on depth and water velocity, temperature, nutrient concentration, and biotic activity. If resources are available, uptake length (see Chapter 8) should be assessed by the solute uptake method prior to choosing the length of the experimental reach.

5. As soon as you have taken your first set of samples (3–7 days after starting the ^{15}N addition), select a type of sample you expect to be quickly labeled (e.g., algae) and submit a "rush" set to an isotope laboratory by express mail. Request a special rapid turnaround. You should first consult with the laboratory manager to determine whether the lab can accommodate rush samples. Plot these data immediately because although these components are unlikely to be at their maximum $\delta^{15}N$ value, the data will show informative distribution patterns. Adjust your sampling

locations or add additional stations if necessary to sample the full travel distance of the tracer.

6. Sample important food web components weekly during the ^{15}N-enrichment period. You might also sample weekly for three weeks *after* the dripper is turned off. The last three weeks of data will show how rapidly stream organisms and detrital compartments lose incorporated N, which is a measure of N turnover in the food web. After sampling is complete, inventory all samples and select your most interesting and complete series for isotope analysis.

7. Plot the δ^{15}N data for each type of sample versus distance downstream and versus time. Can you tell whether primary producers and consumers reached isotopic equilibrium during the experiment? Do the δ^{15}N values of the consumers agree with hypothesized functional-feeding group assignments? Are some organisms unlabeled because they might be detritivores or might feed on allochthonous sources of food?

IV. QUESTIONS

1. Considering your food web diagrams, what types of additional information would you need to quantify linkages between components? How would you go about collecting these data?

2. Does the food web you constructed using stable isotope data differ from that based on gut analyses or functional feeding group designations? Develop testable hypotheses to resolve any discrepancies.

3. How variable are gut analyses for individual detritivores of the same species? Invertebrate predators? Fish? Based on your answers, how many specimens should you collect to get a good idea of the average diet of each of the dominant species sampled?

4. Do dominant consumers show considerable diet overlap, or minimal diet overlap? Answer this question based on gut analyses data, and then answer the same question based on isotope data. If you sampled more than one stream, is diet overlap between consumers similar between streams?

5. If you conducted *Basic Method 3*, did stable isotope plots reflect any point or non-point sources of ^{15}N or ^{13}C? Which food web components reflect anthropogenic sources most strongly?

6. If you conducted *Advanced Method 1*, how far did the dissolved nutrient travel downstream? What species of consumers contain the highest δ^{15}N values? How can you distinguish between detritivores, grazers, and predators? Do all members of a trophic level have similar enrichment in ^{15}N? Do all species have similar shaped distributions in space or in time? Why or why not?

V. MATERIALS AND SUPPLIES

Food Web Diagrams

    Algae, invertebrate, and fish taxonomic keys (see Chapters 16, 20, and 22)
    Dissecting and compound microscopes
    Fine dissecting pins
    Fine-tipped forceps
    Microscope slides and cover slips

    Mounting media
    River samples of detrital and producer components and macroconsumers
    Slide warmer

All Isotope Studies

    Drying oven or freeze dryer
    Glass vials (scintillation vials work well)
    Microfuge tubes (optional)
    4×6 mm tin capsules (optional)
    Stream samples of organic matter components and macroconsumers
    Glass rods to grind up invertebrates and fine organic matter in vials or microfuge
        tubes
    Grinder (e.g., coffee grinder or tissue grinder) for coarse organic matter

Additional Supplies for ^{15}N Enrichment Study

    Discharge data
    NH_4^+ or NO_3^- data from small stream
    NH_4^+ or NO_3^- uptake length data, if resources permit
    Peristaltic pump and battery, or Marriott bottle; tubing
    Labeled ^{15}N such as ^{15}N-NH$_4$Cl

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Section E

Ecosystem Processes

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Primary Productivity and Community Respiration

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I. INTRODUCTION

Primary producers use sunlight as energy to generate organic matter from inorganic compounds and are the most conspicuous base of food webs in the biosphere. Although forms of reduced chemical energy (detritus, DOM) often predominate the organic matter budgets of streams, primary productivity usually makes a significant contribution. Even if that contribution is small, primary producers can be an essential food resource for consumers (Bunn *et al.* 1999). Primary producers in streams include algae, bryophytes (mosses and liverworts), vascular macrophytes, and Cyanobacteria. Other photosynthetic and chemosynthetic bacteria generate organic matter from inorganic nutrients, but they are not considered here because they are found only in specialized habitats and do not produce oxygen.

Primary productivity is defined as the rate of formation of organic matter from inorganic carbon by photosynthesizing organisms and thus represents the conversion of solar energy to reduced chemical energy. Some of this fixed energy is lost through plant (autotroph) respiration (R_a); the portion stored in biomass is termed net primary productivity (NPP), and the total (respired plus stored) is gross primary productivity (GPP). Thus,

$$GPP = NPP + R_a \qquad (28.1)$$

Primary productivity measurement techniques have their basis in the equation for photosynthesis:

$$6 \ CO_2 + 12 \ H_2O \rightarrow 6 \ O_2 + C_6H_{12}O_6 + 6 \ H_2O \qquad (28.2)$$
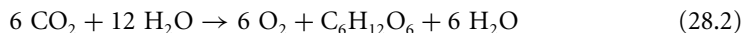
In practice, one measures changes in dissolved O_2 or CO_2 concentrations, with O_2 being most often used, or the rate of uptake of added [^{14}C] bicarbonate, which is used as a tracer of carbon. Gas change procedures allow the determination of both GPP and community respiration. The ^{14}C uptake method provides an estimate of something between GPP and NPP (Vollenweider 1974), regulated in part by environmental conditions and shifting from GPP to NPP with increasing length of incubation (Dring and Jewson 1982). Productivity is a rate and thus measurements carry the units of mass area^{-1} time^{-1} or mass volume^{-1} time^{-1}.

The *in situ* dissolved O_2 change technique was introduced by Odum (1956) and has been used by many others to measure community metabolism in streams (e.g., Flemer 1970, Hall 1972, Fisher and Carpenter 1976, Hornberger *et al.* 1977, Marzolf *et al.* 1994, Mulholland *et al.* 2001). Wright and Mills (1967) used the method in principle but measured CO_2 change. All *in situ* methods are based on the premise that the change in dissolved gas concentration (C) is related to photosynthesis (P), respiration (R), and gas exchange with the atmosphere (E) as long as accrual from surface and groundwater inputs is negligible; thus:

$$C_{(dissolved \ O_2)} = P - R \pm E \qquad (28.3)$$

Instream measurements may be done at one location (single station method), in which case it is assumed that changes in O_2 concentration are identical throughout the reach, or at two stations, one at either end of a study reach (upstream-downstream method), which allows estimation of metabolism as a parcel of water flows downstream.

Two parameters are directly measurable by gas change procedures, net oxygen change in the light (the balance of photosynthesis and respiration) and respiration in the dark. Other metabolic parameters are derived from these data. Respiration measures include the metabolism of heterotrophs (R_h) such as microbes and insects, as well as autotrophs (R_a) and thus are termed community respiration (CR). Measures made over 24 h are analyzed as diel curves (Figure 28.1). Since CR can be determined only in darkness, the average nighttime respiration rate is extrapolated through the daylight hours to generate estimates of photoperiod respiration and total daily respiration (CR_{24}). Although one measures net oxygen change in the light, the estimate is not NPP (as in Eq. 28.1) because, to date, it is not possible to measure the respiration of autotrophs separately. Estimates of GPP, which are generated by adding photoperiod respiration to net oxygen change in the light, also may be subject to error from (1) any asynchrony between photosynthesis and associated metabolic costs (Bott *et al.* 1985) and (2) inability to account for photorespiration (light dependent O_2 uptake and CO_2 evolution accompanied by synthesis of glycolate, some of which is excreted). Net daily metabolism (NDM) is the net O_2 change per day
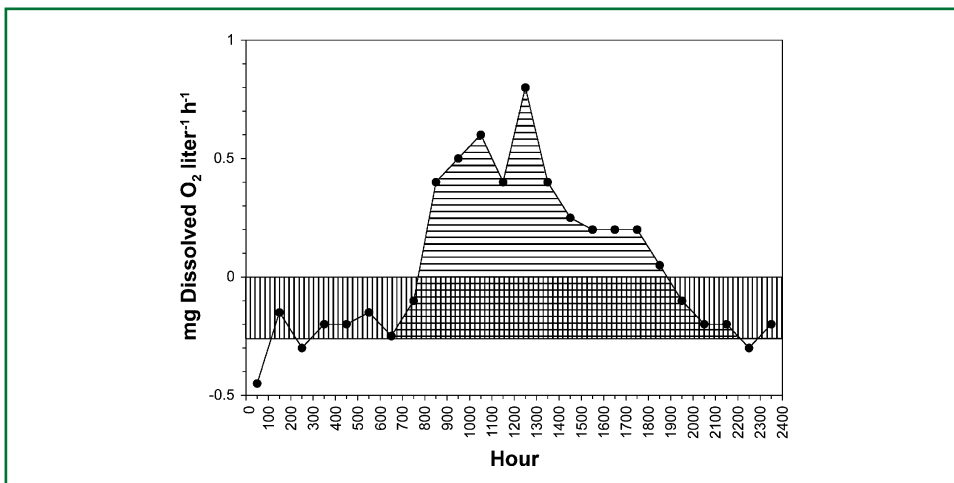
FIGURE 28.1 Rate of change curve derived from changes in dissolved mg O_2 concentration in a respirometer chamber (13 liter water volume) containing a benthic sample (290 cm^2) from Deadman Hole, Salmon River, Idaho, July, 1977. Horizontal lines, GPP, 6.75 mg O_2/liter $\times$ 13 liters $\times$ 1/290 cm^2 = 0.303 mg O_2 cm^{-2} day^{-1}. Vertical lines, CR$_{24}$, 5.52 mg O_2/liter $\times$ 13 liters $\times$ 1/290 cm^2 = 0.247 mg O_2 cm^{-2} day^{-1}.

resulting from biological activity and can be computed as the difference between GPP and CR$_{24}$; thus:

$$NDM = GPP - CR_{24} \tag{28.4}$$

Any error associated with the estimate of photoperiod respiration is removed from the NDM estimate when CR$_{24}$ is subtracted. The P/R ratio (GPP/CR$_{24}$) expresses the balance of these metabolic processes in relative terms. If GPP exceeds CR$_{24}$ for a given 24-h period, there is a net addition of energy to the system, NDM is a positive number and the GPP/CR$_{24}$ ratio is >1. If the reverse occurs, there is a net loss of energy from the system, NDM is a negative number and GPP/CR$_{24}$<1. NDM has been referred to by others as net ecosystem productivity or net community productivity.

Measurements performed directly in the stream determine total system metabolism. In small to midsize streams community biomass is greater in the benthos than in the water column and benthic metabolism dominates system activity. In large rivers and reservoirs planktonic primary productivity and respiration can be greater than benthic sources (Minshall *et al.* 1992), and measurements of water column activity can be done using the light bottle-dark bottle technique (Wetzel and Likens 2000) or respirometer chambers (Minshall *et al.* 1992). In some systems macrophyte productivity may be significant and can be measured using harvest techniques (Westlake 1974).

McIntire *et al.* (1964) introduced the use of respirometer chambers equipped for water recirculation for measuring benthic community metabolism without the need to determine reaeration. In the past 35 years chambers have been used in numerous studies of lotic primary productivity (e.g., Sumner and Fisher 1979, Bott *et al.* 1985, 1997, Minshall *et al.* 1992, Dodds and Brock 1998, Bunn *et al.* 1999). While free from the need to correct

for gas exchange, concerns related to the use of chambers center on nutrient limitation (although chambers have been designed to overcome this, Uehlinger and Brock 1991), alteration of flow regime from that *in situ*, exclusion of hyporheic processes and exchanges, and increases in temperature caused by some pumps. Nonetheless, working with chambers allows researchers to compartmentalize the environment and relate metabolic parameters to the organisms on particular substrata with considerable confidence. However, in order to estimate metabolism of the whole system, it is important to measure the activity on all types of substrata with adequate replication.

Despite widespread use in measuring lentic and marine phytoplankton productivity, the ^{14}C uptake procedure has been used less frequently with benthic stream communities (Naiman 1976, Bott and Ritter 1981, Hornick *et al.* 1981, Hill and Webster 1982, Hill and Dimick 2002). If used, the procedure must include correction for the adsorption of isotope to surfaces and for the proportion of photosynthate excreted by the algae as radio-labeled organic matter. This technique must be applied using closed systems.

The specific objectives of this chapter are to (1) provide researchers with instructions for measuring primary productivity and community respiration and for analyzing data, (2) acquaint researchers with necessary considerations in applying particular methods, and (3) provide criteria used for evaluating the relative importance of these processes in stream system energetics. For additional information concerning methods, consult Vollenweider (1974), Hall and Moll (1975), Wetzel and Likens (2000), and APHA *et al.* (1998).

II. GENERAL DESIGN

A. Site Selection

The study reach should be free of lateral tributaries and significant groundwater input, wadeable, at a stable flow and ideally, have a water surface with minimal turbulence that could alter reaeration characteristics. Reach length may range from <100 m to a few km. The required length of stream for a predetermined change in dissolved gas can be estimated as described by Brock (in the paper by Bott *et al.* 1978). If possible, select a reach for which geomorphologic and hydraulic parameters have already been determined (see Chapters 2 and 3). Third- or fourth-order streams are often used for demonstration of techniques because light reaches the benthos and periphyton communities can be luxuriant. Most pronounced diel changes occur during warm weather in an open reach where large standing crops of algae are found. However, very active communities can rapidly supersaturate water with O_2 and if chambers are used, O_2 is lost to gas bubbles that collect on the lids. Stream selection will be influenced by logistic considerations and the reach should be located in an area safe for personnel making nighttime measurements and for equipment.

B. Methods Selection

Researchers can choose from among several experimental protocols presented below for measuring gas change directly in a stream or in chambers, or for measuring ^{14}C uptake. A problem using real data (Supplementary Exercise) is included to illustrate the principles of data analysis for O_2 change in chambers. Although a few researchers, and even an individual, can carry out a particular protocol, a larger team will allow division of labor for diel measurements if dissolved O_2 is measured using Winkler titrations, for setting up

chambers and, perhaps, for comparing different methods on one stream reach or activity in two or more reaches. In addition to dissolved O_2 determinations (if probes are not used), determinations of water temperature, barometric pressure, reach hydraulic and geomorphologic characteristics, and reaeration are required for the open system method. Chambers are useful for comparing activity on different substrata or for measuring the effect of a change in an environmental variable (e.g., nutrient concentration) on metabolism.

Responses to the following questions will aid in selecting the most appropriate method for the system under study. (1) Is the system nutrient rich with large algal standing crops or nutrient poor with sparse growth? Gas change methods work well with moderate to large algal standing crops, whereas the ^{14}C uptake technique[1] is useful where algal densities are low. Algal responses to light, temperature or nutrient manipulations can be studied with both approaches. (2) Are respirometer chambers with pumps for water recirculation available? For a chamber design, which can be modified as desired, see Bott *et al.* (1978) or Dodds and Brock (1998). (3) If an instream gas change method is to be used, are instream and riparian conditions sufficiently uniform throughout the reach and upstream of it that the single station method could be expected to give reliable estimates? If not, is there a sufficiently long reach with stable reaeration characteristics to apply the upstream-downstream technique? (4) If an instream gas change method is to be used, will reaeration be determined empirically or from reach hydraulic and geomorphic parameters?

The methods are usually applied over diel periods, although shorter times can be used if experiments are conducted in chambers because darkened chambers can be used to provide separate measures of respiration. Likewise, the ^{14}C uptake procedure is generally performed over shorter times using darkened controls to account for heterotrophic uptake and sorption of ^{14}C to surfaces. Dissolved O_2 can be measured using the Winkler titration (Chapter 5) or with probes and meters, some of which have internal data storage capabilities or the capability of being linked to a data logger for continuous monitoring. Before beginning measurements, it is useful to place all probes at a single location in the thalweg of the study stream for 4–5 h (or longer) in order to (1) fine-tune the calibration of each sensor and pair most similar probes for upstream-downstream measurements, or (2) use the data to determine an offset for pairs of probes that can be applied when analyzing data with the upstream-downstream technique. Corollary measurements of photosynthetically active radiation (see Chapter 5), water chemistry (Chapters 9–11; APHA *et al.* 1998), algal biomass or chlorophyll *a* (Chapter 17), and ash free dry mass of detritus (Chapters 12–13) allow metabolism rates to be related to solar energy input, nutrient status, and biomass, and thus aid in interpreting results. If the open system approach is used, the whole-reach measure can be related to reach chlorophyll *a* as follows. First, the occurrence of algal cover types in the reach is determined using a viewing bucket at, for example, a total of 200 locations along several transects evenly spaced through the reach. The chlorophyll concentrations associated with major cover types are determined and weighted for the proportion of reach area of that cover type, and summed for a total reach estimate of chlorophyll. A reach estimate of periphyton organic matter can be arrived at similarly.

Measurements made on the open system require accurate determination of reaeration. The gas exchange coefficient has been determined empirically from the outgassing of SF_6

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[1] Any use of radioisotopes must be in strict compliance with licensing regulations, including those affecting use at field sites. Researchers must be thoroughly familiar with the safe handling of radioisotopes.

(Kilpatrick *et al.* 1989, Wanninkhof *et al.* 1990) and by monitoring O_2 exchange under a nitrogen-filled dome floated on the water surface (Copeland and Duffer 1964). Presently, the most commonly employed approach uses propane evasion (Genereux and Hemond 1992, Marzolf *et al.* 1994, Marzolf *et al.* 1998, Young and Huryn 1998). Other, indirect approaches for assessing reaeration are available if propane evasion cannot be measured. Odum (1956) calculated a diffusion constant from changes in dissolved gas concentrations during predawn and postsunset periods. However, since respiration rates change overnight this method may produce erroneous estimates. Thus, predictions obtained from stream morphometry and hydraulics are preferred because these are independent of metabolic activity. Two options are presented based on different models of reaeration (a) the surface renewal model (SRM; O'Connor and Dobbins 1956, Owens 1974) and (b) the energy dissipation model (EDM; Tsivoglou and Neal 1976, APHA *et al.* 1998).

If chambers are used and periphyton and detritus are fairly evenly distributed in the reach, measures on replicate samples are representative of the reach. However, if periphyton and detritus are unevenly distributed, the best approach is to map their distributions in the reach, measure the metabolism associated with each cover type, and extrapolate to the reach level using the areal proportions of each component.

C. General Basis of Data Analysis

A data sheet is provided for each method, each containing sample calculations. Enter data on similarly designed spreadsheets and analyze as directed. Each method computes hourly changes as the basis for the diel curve and estimation of daily rates. Modify spreadsheets and computations as needed for more frequent sampling. Rates of change in dissolved O_2 concentration are determined for sequential sampling intervals for the single-station or chamber methods, or between upstream and downstream for the two-station method, adjusted for exchange of O_2 with the atmosphere for open system measurements, and integrated over the 24-hr period. Respiration rates for the 24-h period are estimated here by extrapolating the average nighttime respiration value through the daylight hours, but more refined approaches are available (Hall and Moll 1975, Marzolf *et al.* 1994).

Data obtained in O_2 units can be converted to carbon (or energy) units as follows. For photosynthesis, we will assume a photosynthetic quotient (PQ, mol O_2 released during photosynthesis/mol CO_2 incorporated) of 1.2. Then,

$$g \ C \ = \ g \ O_2 \times \frac{1}{PQ} \times \frac{12}{32} \tag{28.5}$$

where $12 =$ the atomic weight of C, and $32 =$ molecular weight of O_2. Conversion to calories (cal) is accomplished using the conversion factor of 11.4 cal/mg C (Platt and Irwin 1973) and to joules using 4.2 joules/cal. For respiration, a respiratory quotient (RQ, mol CO_2 released/mol O_2 consumed) of 0.85 is employed. Then,

$$g \ C \ = \ g \ O_2 \times RQ \times \frac{12}{32} \tag{28.6}$$

Data analysis for the ^{14}C uptake procedure is based on the premise that organisms use ^{12}C dissolved inorganic carbon in the same proportion as the added [^{14}C] bicarbonate tracer. Results are corrected for excreted dissolved organic matter, dark uptake, and sorption of ^{14}C to surfaces.

III. SPECIFIC METHODS

A. Method 1: GPP and CR Determined from Dissolved Oxygen Changes *in Situ*

Determination of Reach Characteristics

Procedures described in Chapters 2–4 may be used to determine reach geomorphologic and hydraulic characteristics. Measurements needed for determining reaeration from propane evasion include reach length, mean width, mean depth, time of travel and discharge. The reaeration coefficient for the surface renewal model (SRM) requires water velocity and depth. The energy dissipation model (EDM) requires discharge, water velocity and the slope of the reach, which can be estimated from a topographic map (7.5 min. series quadrangle). Mean width should be based on at least 10–20 measurements evenly spaced through the reach. Time of travel (T_t) of a parcel of water through the reach is estimated preferably using dye or other conservative tracer because estimates based on velocity meter measurements do not account well for transit through slow pools and at the margins of the reach. Accurate estimates of water velocity and mean depth can be back-calculated from the computer modeling of conservative tracer transport through the reach, using, a streamflow model such as OTIS P (One-dimensional Transport with Input and Storage; Runkel 1998). The model generates an estimate of cross sectional area. When this is divided into discharge, an estimate of water velocity is obtained, and when cross sectional area is divided by width, an estimate of mean depth is obtained. If the modeling approach is not used, one still can compute mean water depth by dividing discharge by water velocity, and then by mean width. Mean water depth also can be calculated by averaging depth measurements made along with width measurements, but the value will not be as accurate as one calculated from discharge.

Measurement of Reaeration from Propane Evasion

Fieldwork. The reaeration coefficient can be based on propane and conservative tracer concentrations in samples collected at only the upstream and downstream stations, or at those locations and additional intermediate stations. Sampling times for the propane experiment are based on a prior experiment in which a pulse of rhodamine WT is visually tracked through the study reach to determine the time of travel of a parcel of water. Discharge for both the rhodamine measure and the propane injection must be similar. The rhodamine WT is added at the planned injection site. The times of first appearance of color (leading edge) and its disappearance (trailing edge) at each sampling station are recorded. The plateau at a station starts at the time of the tail (i.e., last of the old water leaves the station) and ends at a time specified by the sum [leading edge + the planned injection time] (i.e., postinjection water begins to reach the station). When setting sampling times for propane, check those for the bottom station against the anticipated length of the plateau and adjust the duration of the injection to ensure that the plateau is long enough to obtain the desired number of samples. Plan to sample five

times during the plateau, beginning a few minutes after its start and continuing at evenly spaced time intervals.

For the injection, propane is bubbled from a barbecue tank equipped with a regulator and gas diffuser hose (available from aquaculture suppliers) into the stream at the injection site. Simultaneously, a conservative tracer solution (e.g., chloride, bromide, or rhodamine WT) is injected into the stream a few cm upstream of the propane using a peristaltic pump. The propane and bromide are mixed by the bubbling of the propane and by turbulence during transit from the injection site to the upstream sampling station. Locate the injection site far enough upstream from the top sampling station to ensure complete lateral mixing of propane and bromide at the top station, a distance that can be computed according to an equation formulated by Yotsukura and Cobb (1972) and used by Wanninkhof *et al.* (1990). Samples for the conservative tracer analyses (usually 20–25) are collected during the entire injection at the top and bottom stations but only plateau samples need be collected at intermediate stations. Propane samples are collected only during the plateau at all stations. Water samples for tracer are collected into plastic bottles (125 mL) and for propane into 75 mL glass serum vials that are butyl-rubber-stoppered and crimp-sealed in the field. Propane vials must be completely filled and stored under refrigeration. In fast flow it is helpful to collect a water sample into a bucket and fill the vials in the bucket (with care to avoid bubbling). Field blanks are collected at each station. Collect samples of conservative tracer injection solution for analyses. Measure the streamwater temperature during the injection.

Laboratory Analyses. Rhodamine WT is analyzed fluorometrically and chloride or bromide by ion chromatography. If a sufficiently high concentration of chloride is used, conductivity may be used for its analysis (Stream Solute Workshop 1990). For propane analyses, two needles are inserted through the serum stopper and 10 mL of air are injected through one into the crimp-sealed serum bottle to displace an equivalent amount of water through the other and create a headspace. After removing the needles, the bottles are placed on their sides and shaken for 3 h at room temperature to equilibrate propane between the water and headspace. Samples of headspace gases are removed using a gas-tight syringe and injected into a capillary gas chromatograph with a flame ionization detector and helium carrier gas (Marzolf *et al.* 1994).

Computations. The average propane peak area corresponds to 100% at the top station and values may range from <10% to >60% of that at the farthest downstream station. Absolute concentrations are not essential; proportional loss over distance is used to compute the reaeration coefficient. If samples were collected only at the top and bottom stations the propane exchange coefficient can be computed (after Marzolf *et al.* 1994) as:

$$k_{\text{propane}}(1/\text{min}) = \frac{1}{T_t} \ln \left[\frac{G_1/CT_1}{G_2/CT_2} \right] \tag{28.7}$$

where T_t = travel time between stations (min), G_1 and G_2 = plateau propane percentages at upstream and downstream stations, respectively, and CT_1 and CT_2 = plateau conservative tracer concentrations at upstream and downstream stations, respectively, corrected for background. If samples were collected throughout the reach, plot the natural log

of [propane/conservative tracer] ratio at each station against downstream distance and determine the loss rate (k_{length}) as the slope of the line using linear regression. Alternatively, one can use untransformed ratios if a statistical package with nonlinear regression analysis is available, an approach favored by some statisticians. Multiplication by water velocity (m/min) converts this longitudinal loss to a proportion lost per unit time, or k_{propane} (L/min). Multiply k_{propane} by 1.39 to convert to k_{oxygen}. This accounts for molecular size and its effect on diffusion (Rathbun *et al.* 1978). Gas exchange varies with water temperature and it is necessary to account for this if diel variation in water temperature is more than 2–3°C. Adjust the reaeration coefficient to the streamwater temperature (t°C) at each dissolved O_2 measurement time from the average temperature during the propane injection (i°C) by adapting the equation of Elmore and West (1961) as follows:

$$k_{\text{oxygen(t°C)}} = k_{\text{oxygen(i°C)}} \times 1.024^{(\text{t°C}-\text{i°C})} \tag{28.8}$$

Determination of Reaeration from Reach Hydraulics and Geomorphology

Using data obtained in the section on reach characteristics (above) use either Option A or B, or both.

Option A: Determine the mass transfer coefficient from velocity (V, in cm/s) and mean depth (H, in cm) according to the surface renewal model (SRM; Owens *et al.* 1964, Owens 1974) using the following equation:

$$f_{(20°C)} = 50.8 \times V^{0.67} \times H^{-0.85} \tag{28.9}$$

This equation is suitable for streams with velocities from 3 to 150 cm/s and depths from 12 to 335 cm. Wilcock (1982) has examined the applicability of similar equations to systems of specific character. The quantity f is in units of cm/h and is easily converted to m/h or m/min so that it is compatible with other terms in the calculations described later on. If f (a mass transfer coefficient) is divided by mean reach depth, a reaeration coefficient (k) is generated.

Option B: Determine the gas exchange coefficient $K_{2(20°C)}$ at 20°C, from the energy dissipation model (EDM; Tsivoglou and Neal 1976) using the equation:

$$K_{2(20°C)} = K' \times S \times V \tag{28.10}$$

where S is the slope expressed as m/m, V = velocity in m/s; and K' varies with stream flow as in Table 28.1 (APHA, *et al.* 1998). K_2 (1/d) can be converted to a coefficient (1/h) or (1/min) by division.

TABLE 28.1	Estimated Variation in K′ with Stream Flow.
Discharge (m³/sec)	**K′(s m⁻¹ d⁻¹)**
0.028–0.28	28.3×10^3
0.28–0.56	21.3×10^3
>0.56	15.3×10^3
(From APHA et al. 1998.)	

Both f and K are computed for a temperature of 20°C. The equation of Elmore and West (1961) is used to adjust both $K_{2(20°C)}$ and $f_{(20°C)}$ to streamwater temperature at each sampling time as shown for K in equation 28.11. Substitute f for K in Eq. 28.11 as appropriate.

$$K_{2(t°C)} = K_{2(20°C)} \times 1.024^{(t-20)} \qquad (28.11)$$

Diel Curves — Field Protocols

1. At either a single station or at upstream and downstream stations, deploy dissolved O_2 probes in the thalweg for continuous monitoring. Examine the vertical and lateral variability in dissolved O_2 concentrations when positioning the probes.
2. If probes are not available, for a single station curve, collect water samples for dissolved O_2 determinations from the thalweg in triplicate at 1 or 2 h intervals for a 24-h period. For the two-station approach, water samples should be collected close to the travel time of water through the reach, or more frequently. Either collect samples at the same time at each station and adjust for travel time when analyzing the data, or set the downstream sampling time at the upstream sampling time plus the time of travel. Fill BOD bottles with care to avoid introducing bubbles. Measure the water temperature at each sampling time.
3. Determine the dissolved O_2 concentration in water samples using Winkler titrations (Chapter 5) or a probe and meter.
4. Determine the gas exchange coefficient from propane injection or from one of the hydraulic-geomorphologic options.

Data Analysis for a Single Station Study. Use Table 28.2

1. Record field data in columns (Col.) A, B, and C.
2. Calculate the midpoint between each sampling time. This is referred to as the "Time stamp for plot" and is entered in Col. D. Oxygen data will be plotted against that time.
3. Calculate the change in dissolved O_2 concentration (C) for each time interval (e.g., for times t_1 and t_2, $C_{t2} - C_{t1}$,) and enter (with sign) in Col. E.
4. Determine the O_2 saturation concentration (C_S) for each time and enter in Col. F. If not measured by a probe, O_2 saturation status can be determined by comparing dissolved O_2 concentration at a given temperature to a table of saturation values

(found in e.g., Hutchinson 1957, APHA *et al.* 1998) or an O_2 saturation nomogram, some of which account for barometric pressure as well (Wetzel and Likens 2000).

5. If not accounted for by the method used in step 4, adjust saturation concentration (C_S) for barometric pressure if working at elevation >1000 m. Enter the pressure into the numerator of Eq. 28.12. Compute the result and enter in Col. G.

$$C_S \times \frac{\_\_\_\text{mm Hg}}{760\,\text{mm Hg}} \tag{28.12}$$

6. Determine the O_2 saturation deficit or surplus ($C - C_S$) at each sampling time and enter (with sign) in Col. H.

    Calculations for the single station approach are illustrated with reaeration determined from the two hydraulic-geomorphologic models, EDM (follow steps 7–13) and SRM (follow steps 14–19). Instructions are given at the end of this section if reaeration was determined from propane evasion.

Using a Reaeration Coefficient Based on the EDM

7. Adjust $K_{2(20°C)}$ (1/h) for the streamwater temperature at each time using equation 28.11 and enter in Col J.
8. Calculate gas exchange at each time by multiplying the saturation deficit or surplus in Col. H by $K_{2(t°C)}$ (Col. J) using Eq. 28.13 and enter (with sign) in Col. K.

$$\text{gas exchange (mg liter}^{-1}\,\text{h}^{-1}) = (C - C_s) \times K_{2(t°C)} \tag{28.13}$$

    When a deficit exists, O_2 diffuses into the water and gas exchange has a negative sign and when water is supersaturated, O_2 diffuses out of the system and gas exchange has a positive sign. When gas exchange is added to the observed rate of oxygen change, the change due to metabolism is obtained.
9. Average the gas exchange for each hour and enter in Col. L to coincide with the time stamp.
10. Add Col. L to Col. E to obtain the rate of oxygen change corrected for gas exchange and enter (with sign) in Col. M.
11. Multiply data in Col. M by mean reach depth to obtain metabolism estimates in areal units and enter in Col. N.
12. Plot the corrected rate of oxygen change (Col. N) against each time stamp (Col. D). On the plot, set the respiration line by extrapolating the mean overnight respiration rate through the daylight hours. Determine area between the corrected rate of change curve and the respiration line during the photoperiod to obtain GPP. CR_{24} can be calculated as the area between the respiration line and the "0 rate of change" line over 24 h, or by multiplying the average hourly nighttime respiration rate by 24. Areas can be determined from plots on graph paper by summing the number of squares and multiplying by the value of a square ("units on Y axis" × "units on X axis") or using an integration routine available in some statistical packages if the data are analyzed using a computer.

TABLE 28.2 Calculation of Data for a Single Station Curve with Reaeration from the Energy Dissipation Model (EDM) or the Surface Renewal Model (SRM). The table can also be used with reaeration from propane evasion as noted in the text. Recall that mg/liter is equivalent to g/m^3.

A	B	C	D	E	F	G	H
Time of day (h)	Dissolved O_2 Concentration (mg/L)	Temperature (°C)	Time stamp for plot (h)	Dissolved O_2 Rate of change (mg liter^{-1}h^{-1})	Dissolved O_2 Saturation Concentration (C_S, mg/liter)	Dissolved O_2 Sat. conc. @ pressure (C_S, mg/liter)	Saturation deficit/surplus (mg/liter)
00:00	7.00	17.9			9.487	7.802	−0.80
01:00	6.95	17.6	00:30	−0.05	9.545	7.850	−0.90
02:00	7.00	17.4	01:30	0.05	9.585	7.882	−0.88
03:00	6.95	17.0	02:30	−0.05	9.665	7.948	−1.00
...							
...							
...							
22:00							
23:00							
24:00							
	SITE DATA:						
	Mean depth	(m)	0.76				
	Discharge	(m³/s)	24.3				
	Velocity	(m/s)	0.51				
	Slope	(m/m)	0.0032				
	$K_{2(20)}$	(1/day)	24.97				
	k_{oxygen}	1/min					
	f	(m/h)	0.178				
	Barometric pressure	(mm Hg)	675				
	Elevation	(m)	1600				

I	J	K	L	M	N	O	P	Q	R	S
		Reaeration from propane evasion or EDM					Reaeration from SRM			
Temperature adjusted k_{oxygen} from propane	Temperature adjusted $K_{2(t°C)}$ from EDM (1/h)	Gas exchange per volume ($g·m^{-3}·h^{-1}$)	Ave. gas exchange per volume ($g·m^{-3}·h^{-1}$)	Reaeration corrected rate of change per volume ($g·m^{-3}·h^{-1}$)	Reaeration corrected rate of change per area ($g·m^{-2}·h^{-1}$)	Temperature adjusted $f_{(t°C)}$ (m/h)	Gas exchange per area ($g·m^{-2}·h^{-1}$)	Ave. gas exchange per area ($g·m^{-2}·h^{-1}$)	O_2 rate of change per area ($g·m^{-2}·h^{-1}$)	Reaeration corrected rate of change ($g·m^{-2}·h^{-1}$)
	0.9898	−0.79				0.170	−0.136			
	0.9816	−0.88	−0.84	−0.89	−0.67	0.168	−0.152	−0.14	−0.038	−0.18
	0.9782	−0.86	−0.87	−0.82	−0.62	0.167	−0.148	−0.15	0.038	−0.11
	0.9690	−0.97	−0.92	−0.97	−0.74	0.166	−0.166	−0.16	−0.038	−0.19

TABLE 28.3 **Data Calculations for a Two-Station Diel Experiment with Reaeration from Propane Evasion. The table also can be used with coefficients from the SRM or EDM. Recall that mg/liter is equivalent to g/m^3.**

A	B	C	D	E	F	G	H	I	J
Sampling Time (T_S) (Time of Day) (h)	Upstream Temperature (°C)	Upstream Dissolved O_2 concentration (mg/liter)	Downstream Temperature (°C)	Downstream Dissolved O_2 concentration (mg/liter)	Displaced Downstream Temperature (°C)	Displaced Downstream Dissolved O_2 concentration (mg/liter)	Upstream Saturation Concentration (C_S, mg/liter)	Downstream Saturation Concentration (C_S, mg/liter)	Upstream Saturation deficit/surplus (mg/liter)
00:00	12.22	9.94	12.39	9.81	12.03	9.88	10.72	10.76	−0.78
01:00	12.12	9.98	12.21	9.85	11.88	9.92	10.74	10.81	−0.76
02:00	11.96	10.05	12.03	9.88	11.74	9.95	10.79	10.84	−0.74
03:00	11.78	10.09	11.88	9.92	11.65	9.98	10.84	10.86	−0.75
04:00	11.66	10.12	11.74	9.95	11.58	10.02	10.87	10.88	−0.75
05:00	11.54	10.17	11.65	9.98	11.50	10.05	10.89	10.90	−0.72
06:00	11.44	10.19	11.58	10.02	11.42	10.13	10.91	10.93	−0.72
07:00	11.33	10.24	11.50	10.05			10.94		−0.70
08:00	11.24	10.34	11.42	10.13			10.97		−0.63
	SITE DATA:								
	Reach length	(m)	1275						
	Mean width	(m)	24.3						
	Mean depth	(m)	0.276						
	Barometric Pressure	(mm Hg)							
	Elevation	(m)	415						
	Discharge	(m$_3$/s)	1.19						
	Velocity	(m/s)	0.177						
	Time of travel (T_t)	(h)	2						
	Sampling time interval (T_S)	(h)	1						
	Temperature during propane injection	(°C)	12.52						

K	L	M	N	Reaeration coefficient k (1/min)			R	S	S	U
				from Propane 0.02087	from EDM ———	from SRM —— ——				
Downstream Saturation deficit/surplus (mg/liter)	Average Saturation deficit/surplus (mg/liter)	Average Temperature (°C)	Dissolved O_2 Change (Down–Up) (mg/liter)	k_{oxygen} at stream ave. temperature	$K_{2(t°C)}$ at stream ave. temperature	k_{oxygen} at stream ave. temperature	Total Reaeration flux (g/m^3)	Dissolved O_2 change (reaeration corrected) (g.m^{-3}.T$_t^{-1}$)	Dissolved O_2 change per area per T$_t$ (g.m^{-2}.T$_t^{-1}$)	Dissolved O_2 change per area Per T$_S$ (g.m^{-2}.T$_t^{-1}$)
−0.88	−0.83	12.13	−0.06	0.0207			−2.07	−2.13	−0.59	−0.29
−0.89	−0.82	12.00	−0.06	0.0206			−2.04	−2.10	−0.58	−0.29
−0.89	−0.82	11.85	−0.10	0.0205			−2.01	−2.11	−0.58	−0.29
−0.88	−0.81	11.72	−0.11	0.0205			−2.00	−2.11	−0.58	−0.29
−0.86	−0.80	11.62	−0.10	0.0204			−1.97	−2.07	−0.57	−0.29
−0.85	−0.78	11.52	−0.12	0.0204			−1.92	−2.04	−0.56	−0.28
−0.80	−0.76	11.43	−0.06	0.0203			−1.85	−1.91	−0.53	−0.26
				0.0155						

13. Integration also can be accomplished in a spreadsheet as follows. Average the hourly predawn respiration and postsunset respiration rates in Col. N to obtain a mean hourly CR. Multiply by 24 to obtain total daily community respiration (CR_{24}). Sum the hourly rates of net O_2 change (Col. N) for the photoperiod. Multiply the average hourly respiration rate by the length of the photoperiod (in h) and add the absolute value to the sum of net changes to generate an estimate of GPP. Calculate NDM as GPP $-$ CR_{24}.

Using a Mass Transfer Coefficient Based on the SRM

14. Adjust $f_{(20°C)}$ (m/h) for the streamwater temperature at each time using Eq. 28.11 and enter in Col O.
15. Calculate O_2 exchange by multiplying the saturation surplus or deficit (Col. H) by the temperature-adjusted f (Col. O) and enter in Col. P. Retain the sign.
16. Average the gas exchange for each hour and enter in Col. Q to coincide with each time stamp (Col. D).
17. Multiply the oxygen rate of change (Col. E) by mean reach depth and enter in Col. R.
18. Sum the hourly rate of change (Col. R) and gas exchange (Col. Q). Enter in Col. S.
19. Determine GPP, CR_{24}, and NDM as directed in steps 12–13.

Table 28.2 also can be used with reaeration determined from propane evasion. In that case, multiply k_{oxygen} (1/min) by 60 to obtain a coefficient (1/h). Adjust k_{oxygen} for temperature at each time using Eq. 28.8 and enter in Column I. Calculate gas exchange at each time by multiplying the saturation deficit or surplus (Col. H) by $k_{oxygen(t°C)}$ (Col. I) according to Eq. 28.14 and enter in Col. K. Follow steps 9–13 as for the EDM approach.

$$\text{gas exchange (mg liter}^{-1}\,\text{h}^{-1}) = (C - C_s) \times k_{oxygen(t°C)} \tag{28.14}$$

Data Analysis for the Upstream-Downstream Technique.
Use Table 28.3

The computation of metabolism from the upstream-downstream technique is illustrated with a reaeration coefficient (k_{oxygen}) derived from propane injection. However, Table 28.3 can be used with K_2 from the EDM approach, or a k generated from an f by division as described above. Analysis of data using f without conversion is presented by Owens (1974). Table 28.3 demonstrates hourly changes in a reach with a T_t of 2 h and a sampling time (T_S) of 1 h, but can be used with any sampling time interval.

1. Record field data in Cols. A through E.
2. If sampling times at the downstream station were not offset by travel time of water through the reach at the time samples were collected, account for that now by moving downstream data up an appropriate number of lines in Col. F and G.
3. Determine the saturating dissolved O_2 concentrations (C_S) for the upstream and downstream stations (Col. H and I) as directed in step 4 for the single station analyses. Adjust for barometric pressure (if not accounted for by the method used to determine C_S) if working at significant elevation (>1000 m). Use Eq. 28.12.

4. Compute the upstream and downstream saturation deficit or surplus $(C - C_S)$ and enter (with sign) in Col. J and Col. K, respectively. Average these values for each time stamp to obtain a reach deficit or surplus and enter in Col. L.
5. Average the upstream and downstream temperatures (Col. B and F, respectively) and enter in Col. M.
6. Compute the [downstream–upstream] difference in dissolved O_2 (Col. G – Col. C) and enter the value (with sign) in Col. N. This is the change in one travel time.
7. Enter the reaeration coefficient, k_{oxygen} (1/min), obtained from the indicated technique in the header of column O, P, or Q.
8. Adjust the coefficient k_{oxygen} to the average reach temperature at each time stamp using Eq. 28.8 (if k is from propane) or Eq. 28.11 (if from hydraulic and geomorphologic parameters) and enter in the appropriate column.
9. Compute the total reaeration flux into the reach during one travel time using Eq. 28.15 (Young and Huryn 1998), i.e., Col. L × Col. O × T_t (in min) and enter in Col. R. See comments in step 8 for the single station analyses. This is the total O_2 reaeration flux (g m^{-3}) in one travel time.

$$\text{Reaeration flux} = \text{Dissolved } O_2 \text{ deficit} \times k_{oxygen} \times T_t \qquad (28.15)$$

10. Sum the dissolved oxygen change (Col. N) and reaeration flux (Col. R) to generate a reaeration corrected dissolved O_2 change per unit volume in one travel time and enter in Col. S.
11. Multiply the value in Col. S by reach depth to generate a reaeration corrected dissolved O_2 change per unit area in one travel time. Enter in Col. T.
12. Multiply the data in Col. T by the ratio [Sampling time/Travel time, i.e., T_S/T_t, with both times in the same units] and enter values in Col. U. These data are the reaeration corrected dissolved O_2 change per unit area in one sampling time interval. Note that if T_S equaled T_t these numbers would be the same. Plot the data in Col. U against time intervals in Col. A to generate a rate of change curve that you will integrate to estimate metabolic parameters. The time interval chosen for the x axis can be in any convenient units (e.g., min, h), but it is critical that the time interval used in Col. A and U is the same. In the example here the sampling interval was 1 h, thus the values in Col. U (y axis) have the units g·m^{-2}·h^{-1}.
13. Using an integration method from Step 13 for the Single Station Analysis. Compute GPP, CR_{24} and NDM.

B. Method 2: Metabolism Measurements on Benthic Communities Transferred to Respirometer Chambers

Field Protocols

1. Transfer streambed substrata with minimal disturbance to trays that will fit in the chambers. If possible, do this several weeks prior to the experiment. Place the trays in the streambed so that the sediment surfaces in and out of the tray are contiguous and continue colonization.

2. For experiments, transfer trays or samples of other streambed substrata into respirometer chambers filled with stream water. Place lids on the chambers, submerge in the stream, start pumps, and release all air bubbles. It is convenient to start measures just after sunset because overnight respiration will lower dissolved O_2 concentrations and retard the potential onset of supersaturation the next day.

3. Ideally, dissolved O_2 will be monitored continuously by inserting a dissolved O_2 probe through a port into the water recirculation line. If the probe does not have internal data storage capability, use a data logger (linked to a laptop computer for real-time display if possible).

4. If probes are not available, take three initial (T_0) water samples from the respirometer immediately after completing step 2. Stop the pump, and siphon water from the chamber into BOD bottles for dissolved O_2 determinations and replace with fresh stream water. Note the water temperature. Sample at 2–3 h intervals, using the same procedure.

5. If nutrient depletion is a concern, completely exchange the chamber water with care to avoid loss of biomass. Determine the dissolved O_2 concentration in the new water immediately, replacing only the water removed to do so, continue incubation for another period, and repeat the sampling procedure.

6. Sample for 24 h to generate a diel curve. If a shorter experiment is performed, incubations in the light measure net oxygen production and replicates covered with black plastic measure respiration.

7. At the end of the incubation period, drain the chamber carefully to obtain an estimate of water volume and sample the chamber contents for algal biomass (chlorophyll *a*, see Chapter 17) and detritus standing stock estimates (ash free dry mass, see Chapter 12).

Data Analysis Protocol for Diel Curve in Respirometer. Use Table 28.4

1. Analysis of the diel data matches that for a Single Station Analysis in the open system except there is no need to correct for gas exchange.

2. Enter data in Col. A and Col. B.

3. Enter mid-point between each sampling time as time stamp in Col. C.

4. Determine rate of change in O_2 concentration between successive intervals and enter in Col. D to coincide with time stamp.

5. Plot the rate of change against time stamp. Integrate data as directed in steps 12–13 for the Single Station Analysis. If short-term incubations with covered and uncovered chambers were performed, net oxygen change is measured in the light chamber, CR in dark chamber, and the sum of these provides an estimate of GPP, just as for the light bottle-dark bottle procedure. Multiply data (in mg/liter) by the water volume in the chamber (liters) and divide by the substratum surface area to obtain data in units of mg $O_2 \, cm^{-2} \, d^{-1}$.

C. Method 3. Primary Productivity from ^{14}C Incorporation

Field Protocols

1. Transfer, with minimal disruption, replicate samples of periphyton to screw-capped vials (5- to 15-mL capacity). A cork borer can be used to core samples from

TABLE 28.4	Calculation of Data for a Diel Curve Performed in a Respirometer Chamber.		
A	B	C	D
Time of Day (h)	Dissolved O_2 (mg/liter)	Time Stamp for Plot (h)	O_2 Rate of Change (mg liter^{-1} h^{-1})
00:00	6.90		
01:00	6.45	00:30	−0.45
02:00	6.30	01:30	−0.15
03:00	6.00	02:30	−0.30
…			
…			
…			
22:00			
23:00			
24:00			
Chamber volume	(liters)		
Tray surface area	(cm^2)		

cohesive mats. More flocculent growths can be scraped from a known area, pooled, and aliquots transferred to experimental vials.

2. Use several replicates for each incubation condition. Collect 5–6 additional replicates for chlorophyll *a* analyses (see Chapter 17). If a similar amount of biomass is placed in each vial, data can be analyzed on a per sample basis and these extra samples will provide a reasonable estimate of the biomass in all vials. If this is not the case, an aliquot from each vial should be analyzed for chlorophyll *a* and radioactivity values normalized individually. Experiments can also be performed by transferring streambed substrata into respirometer chambers, in which case periphyton samples are taken at the end of the experiment, pooled as appropriate, mixed thoroughly, and subsampled. Much more isotope is needed for an experiment of this scale.

3. Add site water to nearly fill the vial (e.g., add 9.6 mL water to a 10 mL vial, so that with additions of isotope and formalin the total volume will be 9.9 mL).

4. Cover several replicates with aluminum foil sleeves to completely block the light and use as "dark controls."

5. Add [^{14}C]bicarbonate to each vial to provide a final concentration in the range of 0.01–0.1 μCi/mL and close immediately. Try to make additions to successive vials in a convenient volume, such as 0.1 mL, at regularly spaced intervals (e.g., every 30 s) because incubations will be stopped at the same intervals to keep the incubation time for all vials identical. Set up a table in your field book to note the times of isotope addition to each sample. In addition, note isotope manufacturer, Lot No. and specific activity in Table 28.5.

TABLE 28.5 **Calculations for Determining Primary Productivity from ^{14}C Incorporation. In the example computations, data are handled on a "per sample" basis.**

A	B	C	D	E	F	G	H
Sample	Light or dark incubation	Rep	Biomass DPM-1	Biomass DPM-2	Biomass mean DPM/sample	Total DPM in biomass	Dark vials Mean DPM in biomass
1	L	1	8147	8153	8150	24450	
	L	2	8345	8360	8353	25058	
	L	3	10765	10729	10747	32241	
	D	1	1756	1740	1748	5244	4858
	D	2	1501	1480	1491	4472	
2	L						
	L						
	L						
	D						
	D						
3	L						
	L						
	L						
	D						
	D						
…							
…							
…							

SITE DATA:		ISOTOPE DATA:		SAMPLE PROCESSING AMTS.:	
pH	7.3	Lot No.		Biomass	5 mL
Total alkalinity (mg/liter)	55	Manufacturer		Total volume	15 mL
Temperature (˚C)	19	Specific Activity		Filtrate aliquots	2.5 mL
Area sampled (cm^2)	0.95	μCi/vial	1.0		
Correction factor	0.27	DPM/vial	2.2 x 10^6	INCUBATION TIME:	0.75 h
Available ^{12}C/liter (mg/liter)	14. 85				

I	J	K	L	M	N	O	P	Q
Light vials DPM in biomass (dark corrected)	Excretion DPM–1	Excretion DPM–2	Excretion mean DPM/sample	Total DPM excreted	Total DPM metabolized (dark and excretion corrected)	Percent excreted	Total DPM metabolized /hr	mg [^{12}C] metabolized cm^{-2} h^{-1}
19592	611	609	610	3660	23252	15.7	31003	0.0035
20200	488	485	487	2919	23119	12.6	30825	0.0035
27383	530	525	528	3165	30548	10.4	40731	0.0046

6. Incubate all samples at ambient temperature and light conditions for an estimate of *in situ* productivity or under a range of conditions (e. g. temperature) if a manipulation experiment is performed.

7. Collect stream water samples for pH and total alkalinity determinations. Measure water temperature.

8. Incubate samples for the same length of time, usually between 0.5–4 h.

9. Stop isotope incorporation by the addition of formalin to provide a final concentration of ∼0.4% in each vial. Note time of addition in the table started at step 4. Compute the time interval between isotope and formalin additions and note as incubation time in Table 28.5.

Sample Processing in Laboratory

1. Ordinarily, the entire sample from each light incubation is filtered through a 0.45-μm pore-size membrane filter at a vacuum of ≤ 0.5 atmospheres to collect algae for the determination of incorporated radioactivity. Collect the filtrate in a vial or test tube placed in the filter flask under the filter head for measurement of excreted ^{14}C.

2. Remove the filtrate vial and rinse the filter twice with 5 mL of water and discard rinse water. The filtrates from dark incubations are not collected since photosynthesis was eliminated in the controls.

3. Acidify each filtrate to pH 2.5 with 3% H_3PO_4, and bubble with air for 10 min. in a fume hood to drive off inorganic ^{14}C.

4. Transfer subsamples of filtrate from each sample to liquid scintillation vials and add a suitable scintillation cocktail. Reserve the remaining filtrate for additional subsampling if needed.

5. If levels of incorporated radioactivity are expected to be very high, the following procedure is used. Filter an aliquot of incubation water (must be essentially free of biomass) to collect filtrate for excreted ^{14}C as in Step 1. Remove the filtrate vial from under the filter head. Then add a small volume of water to the remaining incubation water and note the total volume. Mix the suspension and filter replicate small aliquots for determination of incorporated radioactivity, noting aliquot volume. Rinse the filters as described above. Record volumes in Table 28.5.

6. Air dry filters.

7. Expose dried filters to fumes of concentrated HCl for 10 min to remove adsorbed [^{14}C]bicarbonate, which can be a problem with highly active communities or in sites with hard water. Do this in a fume hood.

8. Ideally, combust dried filters in a sample oxidizer and count the combusted sample in a liquid scintillation counter. If a sample oxidizer is not available, digest samples with tissue solubilizer and then add cocktail.

9. Count each sample twice in a liquid scintillation counter, using a two-cycle option.

10. Analyze samples collected for chlorophyll a (Chapter 17). Remember to handle samples as radioactive if they are exposed to ^{14}C.

11. Determine pH and total alkalinity (APHA *et al.* 1998).

Data Analysis. Use Table 28.5

1. Table 28.5 is set up on a "per sample" basis, assuming little variation in biomass among vials. Enter sample identifiers in Cols. A–C.

2. Enter data from the liquid scintillation counter for biomass in Cols. D and E. Average the duplicate counts and enter in Col. F. Calculate the DPM in the whole sample if DPM data were for aliquots and enter in Col. G.
3. Average the biomass data for dark incubations and enter in Col. H.
4. Correct light incubation biomass data for isotope adsorption by subtracting the mean of the dark controls (Col. H) and enter corrected the value in Col. I.
5. Enter data from the liquid scintillation counter for filtrates in Col. J and K. Average these values and enter in Col. L. Extrapolate to the whole sample volume and enter total DPM excreted for each light sample in Col. M.
6. Sum the dark-corrected biomass DPM (Col. I) and excreted DPM (Col. M) to get the total DPM metabolized for each light sample and enter in Col. N.
7. Calculate the percentage photosynthate excreted (Excreted DPM/total DPM metabolized) × 100, or (Col. M/Col. N) × 100, and enter in Col. O.
8. Divide total DPM metabolized (Col. N) by incubation time (h) to generate DPM/h and enter in Col. P.
9. Determine available ^{12}C inorganic carbon from a table of pH, total alkalinity, and temperature (Saunders *et al.* 1962) or from a nomogram (Vollenweider 1974, or Wetzel and Likens 2000).
10. Calculate primary productivity as in Eq. 28.16

$$^{12}C \text{ metabolized cm}^{-2}\,h^{-1} = \frac{\text{total DPM metabolized}}{h} \times \frac{1}{\text{DPM added}} \times \frac{\text{mg}^{12}C \text{ available}}{\text{liter}}$$

$$\times \frac{\text{liters}}{\text{incubation vessel}} \times \frac{1}{\text{cm}^2 \text{ substrate}} \times 1.06 \qquad (28.16)$$

where 1.06 corrects for isotopic discrimination (i.e., the fact that ^{12}C is taken up preferentially over ^{14}C). Enter in Col. Q.

D. Supplementary Exercise: Data Analysis for Dissolved O_2 Change in Respirometer Chambers. Use Table 28.6

1. From data in Table 28.6 determine the midpoint between sampling times and enter as "Time stamp for plot" in Col C. Compute the rate of change in dissolved O_2 concentration between successive intervals and enter in Co. D to coincide with "Time stamp for plot."
2. Determine metabolic parameters as described in step 4 of the data analysis for Method 2.

IV. QUESTIONS

1. How did the choice of method affect your estimate of metabolism in the reach?
2. What methodological considerations had greatest affect on your results?

TABLE 28.6	Data from a Respirometer Metabolism Experiment Suitable for Constructing an O_2 Rate of Change Curve.		
Time of Day (h)	D.O. (mg/liter)	Time Stamp for Plot (h)	O_2 Rate of Change (mg liter^{-1} h^{-1})
00:00	6.60		
01:00	6.45		
02:00	6.30		
03:00	6.10		
04:00	5.90		
05:00	5.75		
06:00	5.60		
07:00	5.50		
08:00	5.55		
09:00	5.80		
10:00	6.25		
11:00	6.75		
12:00	7.55		
13:00	8.30		
14:00	8.80		
15:00	8.95		
16:00	9.00		
17:00	9.05		
18:00	9.10		
19:00	9.10		
20:00	8.70		
21:00	8.40		
22:00	8.20		
23:00	8.00		
24:00	7.80		

Note. A tray (337 cm²) of streambed sediment was placed in a respirometer with 13 liters of water. The photoperiod was from 06:00 h to 19:00 h.

3. If you determined reaeration from a propane injection experiment, how did that value compare with estimates from geomorphology and hydrology? How did oxygen change from reaeration compare with metabolic rates in your system?
4. If you compared methods on the same reach, did they give similar results? If you compared reaches, were the results similar? Why or why not?

5. Why is it that gas change procedures measure net O_2 change? What sources of error are included in GPP estimates from gas change procedures?
6. What are NDM and the GPP/CR_{24} ratio for your system? What does this indicate about the energetics of your system? If GPP does not exceed CR_{24} and the condition persisted for a period of time, what would be required to maintain the system in a steady-state condition?
7. What factors are most likely to affect system productivity on a daily basis, and on a seasonal basis? If your experiments involved manipulations of light, temperature, or nutrients, what effects were observed?
8. What kinds of organisms were the predominant primary producers in the stream(s) you studied?

V. MATERIALS AND SUPPLIES

Dissolved O_2 Monitoring

> Dissolved O_2 probe and meter or chemicals for Winkler dissolved O_2 determinations (see Chapter 5)
> BOD bottles (for Winkler determinations)
> Data logger, laptop computer or strip chart recorder compatible with dissolved O_2 probe and meter for data storage
> Thermometer or recording thermograph
> Waste containers for samples titrated in the field

Instream Method

> Equipment for determinations of reach length, width, and depth (Chapter 2)
> Velocity measurement equipment (Chapters 3–4)
> Viewing bucket
> Algae sampling materials for biomass analyses

Propane Injection

> Rhodamine WT for conservative tracer, time of travel study, or both
> Stopwatches
> Propane, gas tank regulator, gas diffusion tube for reaeration measurement
> Bromide or chloride solution (conservative tracer)
> Peristaltic pump and power supply (battery or generator) for delivery of conservative tracer, or Marriotte bottle
> Bottles for conservative tracer sampling
> Serum vials with rubber stoppers and crimp seals (and crimping tool) for propane sampling
> Bucket for water sampling in fast flows
> Flame ionization capillary gas chromatograph for propane analyses
> Gas-tight syringes for gas chromatograph injection
> Ion chromatograph for bromide or chloride analyses
> Fluorometer for rhodamine WT analyses (if used as conservative tracer and more than visual detection is needed)

Respirometer Method

> Clear acrylic metabolism chambers equipped with pumps for water recirculation and large enough to hold streambed samples
>
> Source of power (line current, generator, or storage battery) depending on pump requirements
>
> BOD bottles (if in-line probe and meter are not used) and tubing for siphon
>
> Graduated cylinders for water volume measurements
>
> Black plastic for short-term respiration measurements
>
> Periphyton sampling materials for biomass analyses

^{14}C Uptake Method

> Cork borer or other periphyton sampling equipment
>
> [^{14}C]bicarbonate to provide a final concentration of not more than $0.1\,\mu\text{Çi/mL}$ in each incubation vial
>
> Screw-capped vials with secure seals for incubation
>
> Syringe and needle for adding isotope
>
> Aluminum foil to cover dark incubations
>
> Formaldehyde
>
> Pipettes, preferably repeat delivery
>
> Side arm filtering flasks and membrane filter holders
>
> Vacuum pump
>
> Flat bottom sample vials for filtrate collection
>
> Forceps for handling membrane filters
>
> Membrane filters (0.2–$0.45\,\mu\text{m}$ pore size)
>
> Plastic backed absorbent paper to protect work surfaces
>
> Plastic gloves and disposable lab coat for protection when handling isotope
>
> Test tube racks
>
> HCl and H_3PO_4
>
> Sample oxidizer
>
> Liquid scintillation counter and vials for samples
>
> pH meter and probe
>
> Reagents and equipment for total alkalinity determination
>
> Waste containers

VI. ACKNOWLEDGMENTS

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Secondary Production of Macroinvertebrates

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I. INTRODUCTION

Secondary production is the formation of heterotrophic biomass *through time* (e.g., Benke 1993). Annual secondary production, for example, is the sum of all biomass produced ("production") by a population during one year. This includes production remaining at the end of the year and all production lost during this period. Losses may include mortality (e.g., disease, parasitism, cannibalism, predation), loss of tissue reserves (e.g., molting, silk, starvation), and emigration. The relationship between production and related bioenergetic parameters can be represented by the familiar equations:

$$I = A + F \tag{29.1}$$

and

$$A = P + R + U \tag{29.2}$$

where I = ingestion, A = assimilation, F = food that is defecated (egestion), P = production, R = respiration, and U = excretion (e.g., Calow 1992). Food that is assimilated thus

contributes to production (P), respiration (R), and excretion (U). Each of these are fluxes (or flows) of materials or energy, with units of mass or energy·area^{-1}·time^{-1}. At the level of the individual, P represents growth, whereas at the level of the population it represents the collective growth of all individuals. Obviously, how much an organism grows depends on how much it eats, but growth also depends on how efficiently that food is converted to new tissue. Two characteristics of an organism's bioenergetics determine this efficiency: assimilation efficiency (A/I) and net production efficiency (P/A). Among stream macroinvertebrates, assimilation efficiency is likely to be the most variable term, ranging from less than 5% for detritivores to almost 90% for carnivores (Benke and Wallace 1980). Net production efficiency for macroinvertebrates shows less variation and is often close to 50%. Thus, a detritivore might convert only 2 to 3% ($\approx 0.05 \times 0.5$) of its food to production, whereas, a predator might convert as much as 45% ($\approx 0.9 \times 0.5$).

Historically, different kinds of units have been used to represent secondary production. Strictly speaking, energetic units are most appropriate (e.g., Kcal·m^{-2}·year^{-1} or KJ·m^{-2}·year^{-1}). However, most studies have used mass units. For studies of macroinvertebrates in particular, dry mass (or ash-free dry mass) is the norm. Carbon units, as in primary production studies (Chapter 27), are rarely used. Nonetheless, standard conversions are available. For example, Waters (1977) suggested using: 1 g dry mass $\approx$ 6 g wet mass $\approx$ 0.9 g ash-free dry mass $\approx$ 0.5 g C $\approx$ 5 Kcal $\approx$ 21 KJ). More recently, Benke *et al.* (1999) presented data showing that 1 g dry mass ranges from 0.91 to 0.96 g ash-free dry mass among major insect orders, but values for mollusks and decapods were higher. They also suggested that 1 g ash-free dry mass (rather than 1 g dry mass) $\cong$ 0.5 g C.

There are now many estimates of annual production for entire communities of stream macroinvertebrates (Benke 1993). These range from $\sim$2 to >100 g dry mass m^{-2}, with the majority of values being $\leq$20 g dry mass m^{-2} (Figure 29.1).

A. Biomass Turnover and the P/B Concept

To appreciate the concept of secondary production, it is important to understand the relationship between production and biomass. Biomass (B) is a measurement of how much living tissue mass for a population is present at one instant in time (or averaged over several periods of time), and its units are mass (or energy) per unit area (e.g., g/m^2) (Benke 1993). Production, on the other hand, is a flow (e.g., g·m^{-2}·year^{-1}). Production divided by biomass (P/B) is therefore a rate, with units of inverse time (e.g., year^{-1}). Since any unit of time can be selected for a rate, we can calculate annual P/B, weekly P/B, daily P/B, and so on. P/B is essentially a weighted mean value of biomass growth rates of all individuals in the population. Alternatively, a *cohort* P/B is defined as production of a population over its life span divided by the mean biomass over this same time period. A convenient property of the cohort P/B is that it has a relatively constant value of about 5 (range usually 3 to 8). Because it is calculated over a variable period of time (i.e., life span), it is a ratio (unitless) rather than a rate.

Annual P/B values of benthic invertebrates were once thought to vary from only about 1 to 10 (Waters 1977), and this is probably still true for several groups. For example, a univoltine population with a life span of one year will have an annual P/B of about 5, almost identical to the cohort P/B; a bivoltine population would have an annual P/B of about 10. However, much higher values (approaching or exceeding 100) have now been shown for at least some of the dipterans and mayflies which have very short development times (Benke 1984, 1998). High P/B values are also possible for meiofauna (see Chapter 18). In contrast, organisms with life spans >5 years can have P/B values <1.
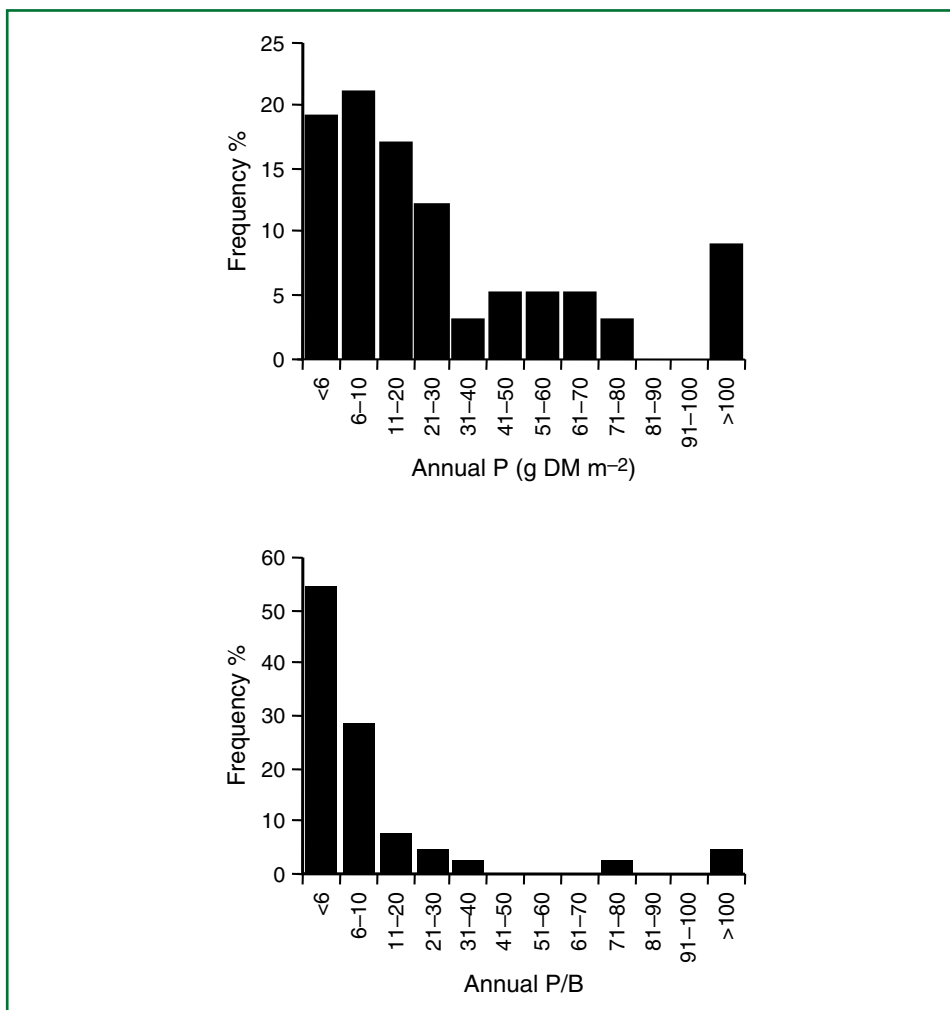
FIGURE 29.1 Frequency distribution of annual production and P/Bs for 58 estimates of benthic macroinvertebrate production for streams and rivers worldwide. Sources and actual values for these estimates are provided in Benke (1993).

Annual P/B values are thus a direct function of the development time of a population and values for individual populations have been shown to vary from <0.1 to >200 (Table 1 in Huryn and Wallace 2000). Annual P/B values estimated for entire communities of stream macroinvertebrates have almost as wide a range from <1 to >100, with most being <6 (Figure 29.1).

B. Utility of Secondary Production in Ecosystem Studies

Understanding factors determining levels and limits of ecosystem production is a central goal in ecosystem ecology. It should therefore not be surprising that studies of secondary production of stream invertebrates have figured prominently in the development of stream ecosystem theory. Since the pioneering monograph by Allen (1951) and the

seminal review by Waters (1977), studies of secondary production in streams have focused on a diversity of ecological questions. These studies fall into three general categories: those simply documenting levels of production of populations and communities (reviews by Benke 1984, 1993, Huryn and Wallace 2000), those attempting to determine physical and biological factors controlling levels and limits to production (Benke 1984, Huryn and Wallace 1987, Huryn 1998), and those that have used estimates of production as a metric for assessing some aspect of the bioenergetic performance of a population or its interactions with other members of its community (e.g., Benke and Wallace 1980, 1997, Ross and Wallace 1981, 1983, Georgian and Wallace 1983, Wallace and O'Hop 1985, Short *et al.* 1987, Plante and Downing 1989, Benke and Jacobi 1994).

Much of the research on secondary production has involved empirically based inductive approaches. However, within the last decade or so, production has been used with increasing frequency as a response variable in experimental studies dealing with specific biotic interactions (e.g., Dudley *et al.* 1990, Vaughn *et al.* 1993), whole ecosystem manipulations (e.g., Wallace and Gurtz 1986, Lugthart and Wallace 1992, Peterson *et al.* 1993), and the effects of land use on stream communities (Sallenave and Day 1991; Shieh *et al.* 2002, 2003, Carlisle and Clements 2003). Estimates of secondary production have been particularly effective in these applications because it integrates a number of other components of ecological performance—density, biomass, individual growth rate, reproduction, survivorship, and development time (Benke 1993).

II. GENERAL DESIGN

Studies of secondary production in streams usually encompass the habitat or reach scale. In the first case, a specific habitat within a study reach is usually sampled (e.g., snag or riffle), and the units of production are reported per area of habitat. In the second, all major habitats within a reach are sampled, and units are reported per area of reach. The appropriate reach length will vary depending upon the purpose of a study, but generally depends on two considerations. The first is habitat structure—all major habitats should be represented in repeated and discrete patches so that variability among habitat patches will be incorporated into the sampling design. Second, the reach length should be long enough to ensure that migration and emigration of individuals during the study will be minimal. For most studies of invertebrate production in wadeable streams, reaches in the range of 50 to 500 m in length are probably sufficient. However, reach length must be considered more carefully in systems where species show migratory behavior, such as freshwater shrimp in the neotropics.

A. Population Density

Estimates of secondary production—regardless of approach—require accurate measurement of population density and size-structure. Sampling methods used to estimate density are usually based on some form of quadrat sampling. Depletion removal methods have also been used for crayfish (Rabeni *et al.* 1997, Whitmore and Huryn 1999). The most appropriate type of sampler will depend on substratum type. A Surber or Hess sampler might be used in a cobble area or on a flat bedrock habitat. A petite ponar grab or corer might work best in shallow gravel or sand (e.g., Ogeechee River corer, Gillespie *et al.* 1985, also manufactured by Wildco, Inc.). As with any quantitative sampling, replication is necessary to obtain accurate density estimates. The distribution of stream

biota is extraordinarily patchy, and this will usually be the greatest contributor to both imprecision and inaccuracy of production estimates. A sufficient number of samples (i.e., from four to six samples have typically been used) should thus be taken to ensure accuracy and to maintain the statistical power of the study to an appropriate level.

Study designs range from completely randomized sampling (habitat and reach scale) to sampling stratified by habitat (reach scale). In the latter case, reach-scale estimates of production can be obtained by calculating production separately for each habitat, quantifying the relative cover of the different habitats, weighting habitat-specific production by the relative area of the habitat, and summing these estimates (e.g., Huryn and Wallace 1987, Smock *et al.* 1992). This latter approach requires fewer samples to attain a given level of precision than completely randomized designs, but requires accurate identification and delineation of habitats.

Most methods used to estimate production require repeated sampling of density over the entire developmental cycle of the target population. Samples are taken monthly in most studies—a schedule that is logical for invertebrates with annual life-cycles. This schedule may be a useful compromise when estimating community production for temperate streams, as well as for many taxa in tropical streams. Monthly sampling will result in poor resolution of the population dynamics of organisms with short life-cycles, however. For studies focusing on such taxa (e.g., *Siphlonisca aerodromia*, Huryn 2002), samples taken at weekly intervals may be required. In cases where growth and development are not synchronous, the sampling schedule is of less concern if steady state biomass can be assumed (see noncohort methods for further considerations for the analysis of such taxa). At the other end of the spectrum, seasonal or even annual sampling may be adequate for long-lived invertebrate taxa (>1 year; e.g., snails, Huryn *et al.* 1997; crayfish, Whitmore and Huryn 1999), as has sometimes been employed for fish production studies (Waters *et al.* 1990).

In temperate streams, short intervals between sampling in spring and summer and long intervals between sampling during winter may be advisable because higher growth rates, and often the bulk of production, typically occur during the warmer months. Caution is required for community studies; however, because some important taxa are bioenergetically active only during winter and early spring. Probably the best approach for accurate estimates of community production is to combine several sampling approaches that are optimal for populations suspected to be major contributors to total system production. Ideally, a thorough knowledge of the life histories of different taxa within a community will allow the planning of a sampling regime that will provide the best accuracy for a given effort, but such information is often not available in advance. If you do not know this in advance, then you can generally obtain general knowledge of expected life histories by searching the literature.

B. Population Size-Structure

Population size-structure refers to the density of individuals within different size classes of a population. For the purpose of estimating production, breaking down a population into size classes is essential for applying methods used in estimating growth and the loss of individuals over time due to mortality, as well as providing a convenient way for estimating biomass. Size classes can be defined arbitrarily on the basis of body length or head-capsule width, or they can be based on criteria such as instar or developmental indicators (e.g., appearance of histoblasts, etc.). The use of length classes is both effective and convenient, however. Length can be measured very precisely using an ocular

micrometer or less precisely using a sheet of 1 mm graph paper placed directly on the microscope stage. The latter approach allows the rapid sorting of individuals into length classes that are suitable for most methods used to estimate production.

C. Individual and Population Biomass

In order to calculate production by any method, it is essential that biomass is determined. The product of length-specific mass (mg) and density (No. individuals/m^2) within a length size-class yields an estimate of size-specific biomass (mg/m^2). The sum of biomass for all size groups is population biomass.

The relationship between individual length and mass for a given taxon is often obtained from a length-mass relationship. Nonpreserved (fresh) animals collected for this purpose provide the best results since preservation (especially in ethanol) results in shrinkage of soft body parts and losses of dry mass by leaching. Animals preserved in a formalin solution will provide estimates comparable to nonpreserved specimens. The procedure involves measuring the lengths of individual animals from a wide range of size categories under a dissecting microscope. The eyepiece must be fitted with a micrometer so that lengths can be measured to at least 0.1 mm. Subsequently, the measured individuals are dried, usually in a drying oven for a minimum of 24 h at 60°C, cooled in a desiccator, and weighed on an analytical balance with acceptable precision. It is best to have at least 20 measurements. A linear regression is then developed of the form

$$\ln W = \ln a + b \ln L \tag{29.3}$$

where $W =$ individual mass, $L =$ length, $a =$ a constant, and $b =$ the slope of the regression. This equation is the linear equivalent of a power curve, $W = aL^b$. Since we expect a cubic relationship between L and W, b should be reasonably close to 3 (Benke *et al.* 1999).

In the absence of time or equipment to determine a length-mass relationship, one can use literature values to obtain length-specific mass. Benke *et al.* (1999) updated and added to the useful equations of Smock (1980) in summarizing relationships for benthic insects, crustaceans, and mollusks from North America, and usually to the genus or species level. Order-level equations from Benke *et al.* are presented in Table 29.1. Additional equations for aquatic insects are presented by Johnston and Cunjack (1999) for northeastern North America; Beerstiller and Zwick (1995), Burgherr & Meyer (1997), and Meyer (1989) for streams in Europe; Towers *et al.* (1994) for invertebrates from New Zealand.

III. SPECIFIC METHODS

The methods for estimating production can be divided into two basic categories: cohort and noncohort (Waters 1977, Benke 1984, 1993). Cohort techniques may be used when it is possible to follow a cohort (i.e., individuals that hatch from eggs within a reasonably short time span and grow at about the same rate) through time. When a population's life history is more complex, a noncohort technique often must be used. Other approaches

| TABLE 29.1 | Mean Values of *a* and *b* From Length-Mass Regressions for Major Insect and Crustacean (Decapoda and Amphipoda) Orders Using Total Length, Except for Decapoda (Carapace Length), Where *W* is Dry Mass (mg) and *L* is Body Length (mm) (Modified from Table 2 in Benke *et al.* 1999). *n* = number of equations from which mean *a* and *b* were obtained. Values of *a* and *b* were not significantly different among insect orders and Amphipoda due to interspecific variability within orders; Decapoda values of *a* and *b* were significantly different from all others. Note that values of *b* are relatively close to 3. |

Order	*n*	*a*	*b*
Decapoda	9	0.0147	3.626
Amphipoda	7	0.0058	3.015
Coleoptera	9	0.0077	2.910
Diptera	43	0.0025	2.692
Ephemeroptera	54	0.0071	2.832
Hemiptera	4	0.0108	2.734
Megaloptera	7	0.0037	2.838
Odonata	18	0.0078	2.792
Plecoptera	36	0.0094	2.754
Trichoptera	34	0.0056	2.839

described below deal with using shortcuts, applying statistical methods, and developing quantitative food webs. Both techniques require quantitative collection (i.e., number per square meter) of the macroinvertebrate species for which estimates are made. See Chapter 20 and Merritt and Cummins (1996) for quantitative collection techniques.

A. Cohort Techniques

As a cohort develops through time, a general decrease in density (N), due to mortality, and an increase in individual mass (W), due to growth, occurs (Figure 29.2). Interval production (i.e., time between two sampling dates) is easily calculated directly from field data by the *increment-summation method* as the product of the mean density between two sampling dates ($\overline{N}$) and the increase in individual mass ΔW (i.e., $\overline{N} \times \Delta W$). Assuming there is only one generation per year, annual production is calculated as the sum of all interval estimates, plus the initial biomass:

$$P = B_{\text{initial}} + \sum \overline{N} \Delta W \tag{29.4}$$

The initial biomass (B_{initial}) represents an approximation of production that has accumulated before the first sampling date.

However, if one wants to examine production patterns throughout the year, mean daily production for an interval can be calculated by dividing each $\overline{N}\Delta W$ by the days in the interval. This converts interval production into a true flow (i.e., $g \cdot m^{-2} \cdot day^{-1}$). A study of the stream caddisfly *Brachycentrus spinae* provides an especially clear example for illustrating the calculation of production using a cohort method, such as the increment-summation method (Table 29.2, modified from Ross and Wallace 1981). Ross and Wallace
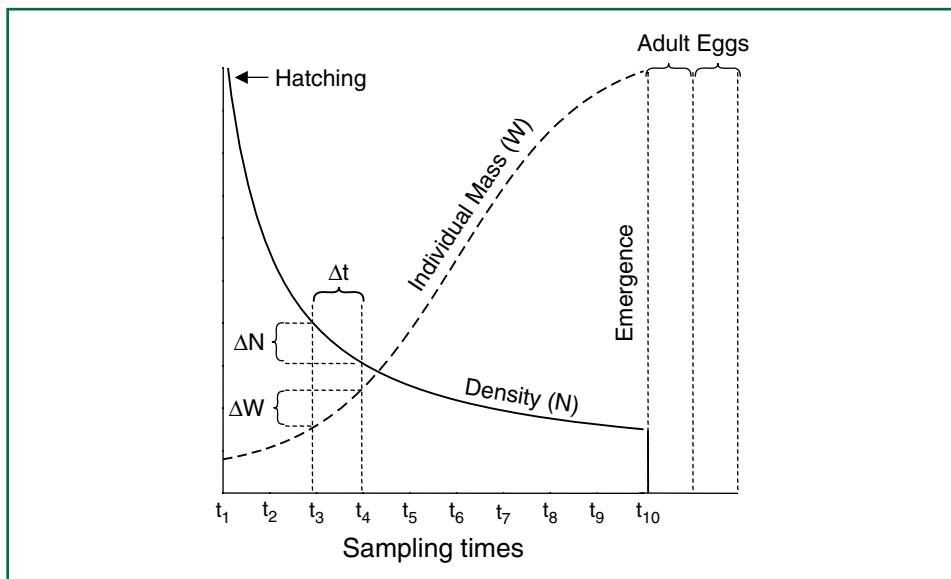
FIGURE 29.2 Hypothetical cohort of a stream insect showing curves of individual growth in mass (*W*) and population mortality (*N*) (modified after Benke 1984).

(1981) used the *instantaneous growth method* (see following), which provides production estimates very similar to those in Table 29.2. Note that the cohort P/B ratio is close to 5 (i.e., 5.96).

In addition to the increment-summation method, there are three closely-related ways of calculating production using cohort data that should give very similar results (e.g., Waters 1977, Gillespie and Benke 1979, Benke 1984). The *removal-summation method* is most similar to the increment-summation method, but it calculates production *lost* during the sampling interval as the product of the decrease in density (ΔN, Figure 29.2) and the mean individual mass ($\overline{W}$) over the interval (i.e., $\overline{W} \times \Delta N$ rather than $\overline{N} \times \Delta W$). Adding the increase in biomass (ΔB) between sampling dates to the production loss equals interval production as calculated above (i.e., $\overline{W}\Delta N + \Delta B = \overline{N}\Delta W$ for any interval). The *Allen curve method* is a graphical approach (Figure 29.3) in which the area under a curve of density vs. mean individual mass approximates total production of a cohort (Allen 1951, Waters 1977, Gillespie and Benke 1979, Benke 1984). The Allen curve also illustrates the relationship between biomass (B), and the changes in numbers (ΔN) and individual mass (ΔW) over sampling intervals that are used in the tabular methods (e.g., Table 29.2). For example, $Y + Z$ (Figure 29.3) $\cong \overline{N}\Delta W$ in the increment-summation method (Table 29.2). The *instantaneous growth method* can also be used to calculate production during a sampling interval (see below).

B. Noncohort Techniques — Size-Frequency Method

When a population cannot be followed as a cohort from field data, it is necessary to use a noncohort method to estimate production. These methods require independent approximations of either development time or biomass growth rates. The *size-frequency*

TABLE 29.2	Calculation of Annual and Daily Production of *Brachycentrus spinae* (Data from Ross and Wallace 1981) Using the Increment-Summation Method.

Date	Density (No./m²) N	Individual Mass (mg) W	Biomass (mg/m²) $N \times W$	Individual Growth (mg) $\Delta W = W_2 - W_1$	Mean $\overline{N}$ (No./m²) $(N_1 + N_2)/2$	Interval P (mg/m²) $\overline{N}\Delta W$	Daily P (mg m^{-2}d^{-1}) $\overline{N}\Delta W/\Delta t$
18 May	282.9	0.021	5.9				
				0.036	254.9	9.17	0.66
1 Jun	226.8	0.057	12.9				
				0.031	204.4	6.33	0.53
13 Jun	181.9	0.088	16.0				
				0.085	160.2	13.62	0.85
29 Jun	138.5	0.173	24.0				
				0.179	123.9	22.17	1.58
13 Jul	109.2	0.352	38.4				
				0.588	98.4	57.83	4.45
26 Jul	87.5	0.940	82.3				
				0.266	73.9	19.64	0.89
17 Aug	60.2	1.206	72.6				
				0.590	54.3	32.01	2.46
30 Aug	48.3	1.796	86.7				
				0.025	42.6	1.06	0.07
15 Sep	36.8	1.821	67.0				
				1.378	32.0	44.03	2.45
3 Oct	27.1	3.199	86.7				
				0.358	20.1	7.18	0.17
14 Nov	13.0	3.557	46.2				
				1.074	10.9	11.71	0.51
7 Dec	8.8	4.631	40.8				
				2.222	6.2	13.83	0.27
27 Jan	3.8	6.853	26.0				
				1.624	3.1	5.08	0.24
17 Feb	2.6	8.477	22.0				
				3.071	2.2	6.60	0.28
13 Mar	1.7	11.548	19.6				
				3.252	0.9	2.76	0.06
27 Apr	0.0	14.800	0.0				
				Annual P	$=5.9+$	253.03	$=258.93$

Cohort B$=43.4$ Cohort P/B$= 5.96$

Annual B$= 39.8$ Annual P/B$= 6.51$

Note: P, production; B, mean biomass; $\overline{N}$, mean density between two consecutive dates. Annual production is calculated by adding the sum of the interval production column and the biomass estimated on the first sampling date. Mean biomass was estimated from monthly means since the sampling regime involved both monthly and bimonthly samples. Thus, mean cohort biomass was for 11 months and mean annual biomass for 12 months.

method (Hynes and Colemen 1968, Hamilton 1969, Benke 1979) assumes that a mean size-frequency distribution determined from samples collected throughout the year approximates a mortality curve for an *average cohort*. A study of the stream mayfly *Tasmanocoenis tonnoiri* provides a good illustration of this method (Table 29.3, modified from the data of Marchant 1986). The decrease in density (ΔN) from one size (i.e., length) category to the next is multiplied by the mean mass between size categories ($\overline{W}$), using the same rationale as for the removal-summation method. Before summing the products (i.e., $\overline{W}\Delta N$) for each size class, each value should be multiplied by the total number of size classes (Table 29.3, final column). This is done because it is assumed that there is a total development time of one year, and that there is the same number of cohorts during the year as
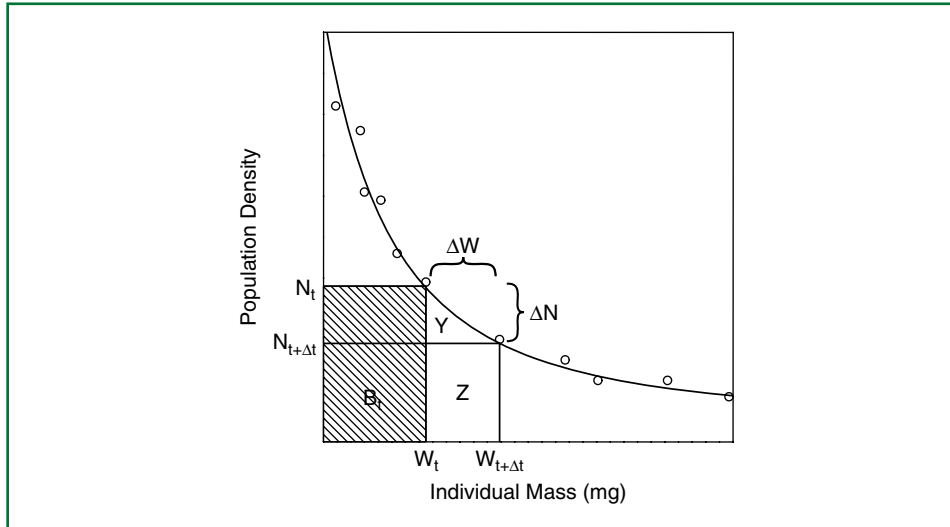
FIGURE 29.3 Hypothetical Allen Curve for estimating production. Circles indicate means of density and individual mass from samples. Curve is smoothed to provide an approximate fit to the points. Production is equal to the area under the curve. Note that W_t, N_t, ΔW, ΔN, and B_t correspond to the same terms in Table 29.2.

size classes (see Hamilton 1969 or Benke 1984 for a more complete rationale). Cohort P/B is equal to the sum of the biomass column (i.e., the true mean annual biomass) divided by the sum of the final column (i.e., production assuming a one-year life span). In this particular case, the cohort P/B (9.5) is considerably higher than usually expected (5), due to the fact that a very small fraction of the population survived to the larger size classes.

If development time is much different than a year, it is necessary to apply a correction factor to the basic size-frequency calculation; sum of final column of Table 29.3. This involves multiplication by 365/CPI where CPI (i.e., cohort production interval) is the mean development time in days from hatching to final size (Benke 1979). In the example of Table 29.3, Marchant estimated a mean CPI of five months based upon his interpretation of life histories from size-frequency histograms. Annual production is thus calculated, using months rather than days, as $352.5 \times 12/5 = 846.1 \ \mathrm{mg \cdot m^{-2} \cdot year^{-1}}$, with an annual P/B of 22.9. These estimates are somewhat different than found by Marchant since he used a geometric rather than a linear calculation of mean individual mass between size categories (mass at loss, sixth column of Table 29.3). Some investigators argue that geometric means provide more accurate estimates of individual biomass over a given time interval because growth is usually exponential rather than linear. The use of geometric vs. linear means is usually a matter of preference, however, because the former generally provides only slightly lower values than the latter. Shorter CPIs (e.g., 30 days) require even greater corrections (i.e., $365/30 \cong 12$). If it is not possible to approximate CPI from field data, as done by Marchant, it is necessary to obtain this information from populations reared in the laboratory or in the field. A final point is that CPI is inversely related to biomass turnover rates (i.e., daily or annual P/B). For example, if CPI = 30 days (a relatively short time), then annual P/B = cohortP/B $\times 365/\text{CPI} \approx 5 \times 365/30 \approx 60$ (a relatively high value). Benke (1993) noted that the *size-frequency method* has been used more than any other production method for stream invertebrates and this is probably still true.

TABLE 29.3	Calculation of Annual Production of *Tasmanocoenis tonnoiri* (Data from Marchant 1986) Using the Size-Frequency Method.

Length (mm)	Density (No./m²) N	Individual Mass (mg) W	No. Lost (No./m²) ΔN	Biomass (mg/m²) $N \times W$	Mass at Loss (mg) $\overline{W} = (W_1 + W_2)/2$	Biomass Lost (mg/m²) $\overline{W}\Delta N$	Times No. Size Classes $\overline{W}\Delta N \times 6$
0.5	706.0	0.001		0.71			
			−142.0		0.011	−1.491	(−8.95)[a]
1.5	848.0	0.02		16.96			
			730.0		0.050	36.5	219.00
2.5	118.0	0.08		9.44			
			72.0		0.130	9.36	56.16
3.5	46.0	0.18		8.28			
			42.0		0.265	11.13	66.78
4.5	4.0	0.35		1.40			
			3.7		0.435	1.61	9.66
5.5	0.3	0.52[b]		0.16			
			0.3		0.520[b]	0.16	0.94

	Biomass = 36.94	Production (uncorrected) = 352.5
	Cohort P/B = 9.5	
	Annual P/B = 22.9	Annual P[c] = 846.1 (Prod. × 12/5)

Note: The density column (the average cohort) is the mean value from samples taken throughout the year. P, production; B, mean biomass; $\overline{W}$, mean individual mass between two size classes
[a] *Negative value at top of table (right column) disregarded, since it is probably an artifact caused by inefficient sampling of smallest size class or rapid growth through size interval. If negative values are found below a positive value (not shown in example), they should be included in the summation.*
[b] *Final "mass at loss" should be equal to individual mass of the largest size class.*
[c] *Annual production is calculated by multiplying "uncorrected production" by a CPI correction factor (12 mo/CPI), where CPI = 5 mo (see text).*

C. Noncohort Techniques—The Instantaneous Growth Method

The second noncohort technique is the *instantaneous growth rate method*. It involves the calculation of a daily instantaneous growth rate:

$$g = \frac{\ln(W_{t+\Delta t}/W_t)}{\Delta t} \tag{29.5}$$

where W_t = mean mass of an individual at time t, $W_{t+\Delta t}$ = mean mass of an individual at time $t+\Delta t$, and Δt = length of the time interval. Daily production (P_d) is calculated as:

$$P_d = g \times \overline{B} \tag{29.6}$$

where $\overline{B} =$ mean population biomass for two consecutive dates in units of g/m² (e.g., Benke and Parsons 1990, Benke and Jacobi 1994). Unlike the size-frequency method, the instantaneous growth rate method is valuable for tracking changes in production over time (e.g., Georgian and Wallace 1983, Benke 1998).

When applying the instantaneous growth rate method as a *cohort* approach, *g* may be estimated directly from changes in average cohort-biomass between sampling dates using Equation 29.5. The effect of sample error on estimates of growth rate between sampling intervals, which may result in negative values for *g* if growth rates are low, may be reduced by regressing mean individual mass against days since hatching using a continuous exponential model to estimate W_t and $W_{t+\Delta t}$. Another potential source of error when using this approach occurs late in cohort development of aquatic insects when apparent mean-size decreases due to the early emergence of large individuals. Unless this latter source of error is accounted for, the sample method results in underestimates of *g*, which can lead to large errors in production because population biomass is often greatest shortly before emergence.

When using the instantaneous growth method as a *non-cohort* approach, g is estimated from animals grown in the laboratory or in the field (e.g., Huryn and Wallace 1986, Hauer and Benke 1987, 1991). For very large invertebrates, such as snails and crayfish, growth rates can be measured using mark and recapture of free-ranging individuals (branding, tagging, tattooing; Huryn et al. 1995, Whitmore & Huryn 1999). An alternative procedure that can be used for many different taxa is through the use of *in situ* growth chambers. Larvae of various sizes are confined in chambers, such as can be made from short lengths of plastic tubing (i.d. = 7.7 cm) capped with 63 to 500 μm mesh. The mesh can be attached by gluing or with cable ties. The fine mesh has been used for chironomid larvae (Huryn and Wallace 1986), the larger for mayflies (Leptophlebiidae, Siphlonuridae; Huryn 1996a, 2002). Chambers may be anchored directly to the stream bottom. In habitats where oxygen may reach low levels (e.g., floodplain swamps), they should be supported on foam floats in such a way that a portion of the mesh on either end of the chamber will be submerged regardless of fluctuating water levels (Huryn 2002). Chambers may be loosely packed with conditioned detritus or pebbles coated with biofilm to provide food (Huryn 1996, 2002). Alternatively, the chambers may be deployed for two to three weeks prior to stocking to allow biofilm to grow on their walls. All of these approaches have been used successfully in both streams and wetlands (Huryn 1996a, 2002, and unpublished). Once chambers are prepared, individual larvae or groups of even-sized larvae, representing the range available for a given taxon, are measured and placed into the growth chambers. At appropriate intervals (e.g., weekly), larvae are removed, their lengths recorded, and new individuals placed in the chambers. *g* is calculated using Equation 29.5 and further equations estimating *g* as a function of water temperature and individual mass may be derived using regression models (e.g., Huryn and Wallace 1986, Hauer and Benke 1987, 1991). In cases where both the cohort and noncohort approaches can be applied simultaneously, the accuracy of growth rates obtained from confined individuals may be assessed by comparing plots of predicted growth trajectories based on the regression equations, to size-frequency data from the field (Huryn 2002).

The most accurate estimates of production will probably be obtained when it is possible to determine size-specific growth rates due to differences in growth rates between size

classes. Production of the *i*th size class is $P_i = g_i \times \overline{B}_i$, where g_i and $\overline{B}_i$ are the growth rate and mean biomass of the *i*th size class, respectively, and total daily production is the sum of the production of all size classes,

$$P_d = g_1 B_1 + g_2 B_2 + \cdots + g_i B_i \qquad (29.7)$$

D. "Shortcut" Approaches

Given the amount of labor required to directly measure invertebrate production, it is not surprising that shortcut approaches have been developed. Of the several offered (Benke 1984), we believe that three are particularly useful. The first is based on the annual P/B, which is a rough estimator of the annual biomass growth rate of a population. Waters (1969) showed that the cohort P/B values for invertebrates populations fell within a relatively narrow range (2 to 8), and suggested that *cohort production* might be estimated as the product of biomass and a suitable P/B (5 is usually suggested). Thus, if a population is known to be univoltine, an annual P/B of 5 can be used as an approximation. When entire benthic communities are considered, however, the range of P/Bs for *annual production* varies from <1 to >100 because cohorts of different taxa may require periods of several weeks to several years (Benke 1993). The range of expected annual P/Bs can be narrowed by considering only small temperate streams (e.g., mean discharge ~0.1 to 1.0 m³ s⁻¹ and mean annual temperature 5–10°C), where annual P/B's range from 2.2 to 8.7 (10 streams) or from 4.2 to 7.9 (7 streams) (Benke 1993). On the basis of these prior studies, a rough but reasonable estimate of the expected range of secondary production for a stream with similar characteristics can be obtained as the product of community biomass and a range of annual P/Bs from 2.2 and 8.7 or even from 4.2 and 7.9.

A second useful shortcut approach is based upon meta-analysis using multiple regression models to estimate invertebrate production as a function of more readily measured variables, usually temperature, population biomass, and maximum body size (Morin and Bourassa 1992, Benke 1993, Morin and Dumont 1994, Benke *et al.* 1998). Empirical models now seem to be used quite frequently for marine benthos (Brey 1990, Tumbiolo and Downing 1994). However, this approach has been criticized because it is prone to imprecision and inaccuracy, particularly when used to estimate production for single species at a single location (Benke 1993). On the other hand, the estimation of production for entire communities or groups of species (e.g., functional groups) as summed composites of estimates for single populations may increase the accuracy of this approach (Benke *et al.* 1998). Indeed, Benke (1993) and Webster *et al.* (1995) showed that production of invertebrate functional feeding groups (sensu Cummins 1973) may be estimated as various linear and polynomial functions of stream size (as indicated by mean annual discharge). Although the latter models are not appropriate for estimating production per se, the testing and refinement of meta-analytical approaches merits further research because they may provide biologically reasonable ranges of production at large spatial scales required for multiscale studies of stream ecosystem processes (e.g., among general categories of streams).

A third shortcut method that appears to hold promise is the use of the biomass of emergent aquatic insects as an indicator of total larval production. A meta-analysis by

Statzner and Resh (1993) revealed a statistically significant relationship between emerging biomass and benthic secondary production for 18 streams in Europe ($r^2 = 0.81$, $p < 0.0001$). Their analysis indicated that adult emergence represented $\sim$24.3% of benthic insect production for these streams. A useful "rule of thumb" for estimating stream insect production for the region represented in the analysis would thus be product of the biomass of emerging insects and "4.1." (i.e., 1/0.243).

E. Statistical Approaches

The quantification of the uncertainty of production estimates has been a long-standing problem, due primarily to the singular nature of production studies. For example, a study of community production for a stream will ultimately provide a single value. The uncertainty of this value, and thus the ability to objectively compare it to values estimated for other systems, however, will be unknown. This is because methods used to estimate such uncertainty require replication. In most studies of lotic secondary production, the appropriate replicate is the stream itself. Unfortunately replication at this spatial scale will be impossible in many, if not most, cases. Several attempts to produce algorithms estimating variance for production estimates have been suggested (Krueger and Martin 1980, Newman and Martin 1983, Morin *et al.* 1987). The most flexible method, requiring the fewest assumptions is bootstrapping—a nonparametric resampling technique (Effron and Tibshirani 1993).

Bootstrapping is used to estimate the uncertainty of variables with unknown or complex frequency distributions and for situations in which logistical constraints do not allow replication. At minimum, it provides an estimate of the uncertainty inherent in a particular data set. If the data are unbiased and of sufficient coverage, however, bootstrapping will provide an estimate of the true probability distribution underlying any given parameter (Effron and Tibshirani 1993). It is important to be aware that, as for any statistical approach, bootstrapping requires a solid foundation of data for meaningful results — it also requires the same philosophical and methodological rigor as other statistical approaches.

Bootstrapping has been used to estimate confidence intervals for production estimates for both populations and communities calculated by both the size-frequency and instantaneous growth methods (Morin *et al.* 1987, Huryn 1996b, 1998). Estimates of confidence intervals (CI) are derived by randomly resampling each of the original data sets used to estimate production, with replacement, until a predetermined number of bootstrap data sets are produced (usually 500 or 1000). The mechanical process of randomizing and iteratively resampling the original data sets to produce bootstrap data sets is readily accomplished using Microsoft Excel© spreadsheet functions and Visual Basic©. The bootstrap data sets are then combined to estimate production which ultimately yields a vector of bootstrap production values (usually 500 or 1000). A mean and approximate 95% CI — as an example — can then be produced from this vector by discarding the upper and lower 2.5% of bootstrap values (Figure 29.4) or by using an alternative approach such as the bias-corrected percentile method (Meyer *et al.* 1986). Differences between two vectors of bootstrap production values can be assessed by comparing the degree of overlap of confidence intervals or by using an approach such as the two-sample randomization test (Figure 29.4; Manly 1991). In cases where more than two vectors are involved, preplanned orthogonal comparisons can be assessed using a matrix of probabilities estimated using the two-sample randomization test. Family-wise error rate can be controlled using the Bonferroni correction (Keppel 1982).
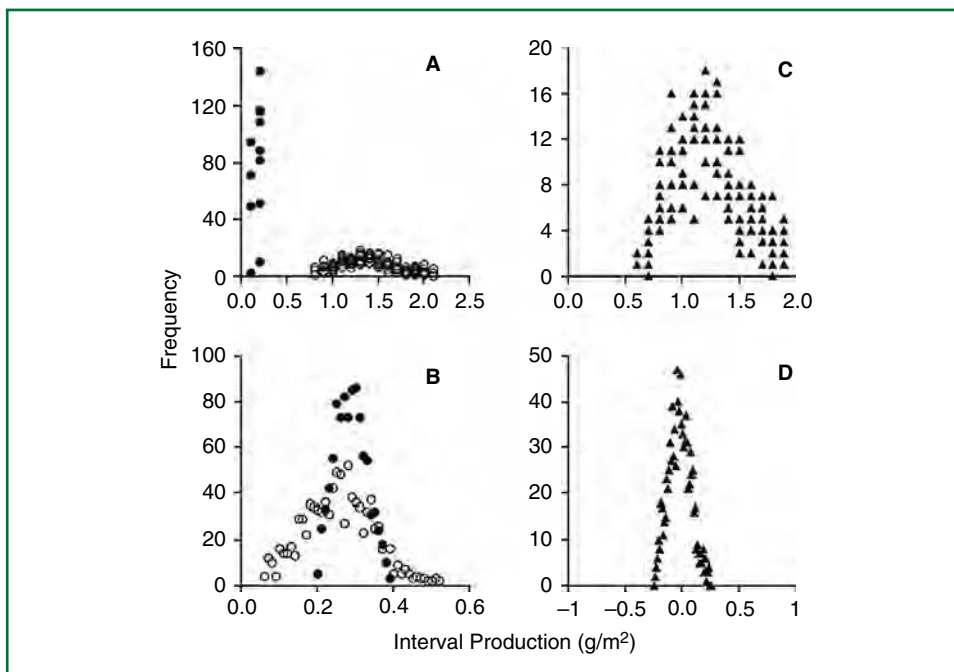
FIGURE 29.4 A. Results of a bootstrap analysis of production by *Siphlonisca aerodromia* in a floodplain wetland in Maine in 1997 (filled circles) and 1998 (open circles, Huryn 2002 and unpublished). The frequency distribution of the bootstrap data set for each year is shown. Only points falling within the 95% CI are included (i.e., the highest 25 values and the lowest 25 values of 1000 total are not shown). The lack of overlap between 95% CIs indicates that production was significantly different between years. B. Same as A except that the data are for *Eurylophella* and production between years is not significantly different. C. Application of the two-sample randomization test to data shown in A. The bootstrap vector for production in 1997 was subtracted from 1998 and the 95% CI of the resulting frequency distribution is shown. The 95% CI does not contain zero, indicating that production between years was significantly different. D. Same as C except that the data are for *Eurylophella* and the 95% CI contains zero, indicating that production between is not significantly different between years.

F. Quantification of Food Webs

An application of production analysis is in the quantification of food webs, a subject of considerable interest in ecology. Although there are several approaches to this topic, a frequently asked question concerns the strength of linkages among members of a community. One method for quantifying food web linkages employs production analysis, gut analysis, and energetic efficiencies (Benke and Wallace 1980, 1997). Given production of an individual population and quantitative data on its gut contents, it is possible to determine the amount of food eaten by that population if the ecological efficiencies of food types can be approximated. For example, production and consumption for the filter-feeding caddisfly *Arctopsyche irrorata* is illustrated in Table 29.4 (from Benke and Wallace 1980), showing that it is primarily a predator. If this can be done for most of the major consumers in a stream ecosystem, then quantitative food webs can be constructed which show the relative amounts of each food type consumed as well as the "trophic position" of each species (e.g., Benke and Wallace 1997, Hall *et al.* 2000, Benke *et al.* 2001, Stagliano

| TABLE 29.4 | Procedure for Calculating Production Attributed to Each Food Type and the Amount of Each Food Type Consumed for the Stream Caddisfly *Arctopsyche irrorata* (Annual Production = 605 mg m^{-2} yr^{-1}). (From Benke and Wallace 1980.) | | | | | | | | | | | | |

	Food Type in Foregut (%)		Assimilation Efficiency (AE)		Net Production Efficiency (NPE)		Relative Amount to Production	Production Attributed to Food Type (%)	Production Attributed to Food Type (mg m^{-2} y^{-1})		Gross Production Efficiency (AE × NPE)		Amount Food Type Consumed (mg m^{-2} y^{-1})
Animal	73.1	×	0.70	×	0.5	=	25.6	93.3	564	÷	0.35	=	1611
Vascular plant detritus	4.2	×	0.10	×	0.5	=	0.21	0.8	5	÷	0.05	=	100
Fine detritus	17.9	×	0.10	×	0.5	=	0.90	3.3	20	÷	0.05	=	400
Filamentous algae	2.0	×	0.30	×	0.5	=	0.30	1.1	7	÷	0.15	=	47
Diatoms	2.9	×	0.30	×	0.5	=	0.44	1.6	10	÷	0.15	=	67

and Whiles 2002). Such an approach can be extremely useful in functional analyses and determining major pathways of energy flow, rather than just "connectivity webs."

IV. QUESTIONS

1. What is a reasonable value for annual P/B if you have a population with two generations per year? For a population with a five-year life span?
2. When using a cohort table for estimating production (e.g., Table 29.2), do you obtain mean annual biomass by adding or taking the average of values in the biomass column? How about when you use a size-frequency table calculation (e.g., Table 29.3)?
3. When adding the final production column for either the cohort table or the size-frequency table, do you include or exclude negative values from your summation?
4. Approximately what value do you expect to find for the exponent in a power curve that predicts individual dry mass from body length? What does this tell you about a population's growth rate?
5. The "Allen paradox" represents a situation where there does not appear to be enough invertebrate biomass to satisfy the energetic needs of a predator (either a fish or invertebrate predator). How might high values of the P/B ratio help resolve this paradox? What other explanations might there be for the paradox?
6. Why might secondary production be a better response variable for comparative or experimental studies than density or biomass?
7. Why is it necessary to use a correction factor (CPI) in the size-frequency method if the development time is much less than a year? Will you obtain the same annual P/B for two univoltine populations, one of which completes its development in 6 months (i.e,. 6 months of zero biomass) and one of which completes development in 12 months?
8. From the data in Table 29.2, calculate daily production with the instantaneous growth rate method. How does it compare to the increment-summation estimate?

V. MATERIALS AND SUPPLIES

Field Materials

    Buckets (sturdy, 20-L, with lids)
    Coarse brush
    Forceps
    Plastic bags for temporary storage of sample (size of bag depends on sampler size)
    Preservative (ethanol or formalin) stained with Phloxine B or Rose Bengal
    Sampler for quantitative sampling (see Chapter 20)
    Sieves (if samples partially processed in field)
    Growth chambers + microscope for measuring animals

Laboratory Materials

    20 mL scintillation vials, or equivalent (for storage of sorted samples)
    100–500 mL jars (for storage of unsorted samples)
    70% ethanol

   Fine dissecting forceps
   Shallow dishes (Petri dishes)

Laboratory Equipment/Supplies

   Analytical balance (optional)
   Desiccator (optional)
   Dissecting binocular microscope, light source (fiber optic) and ocular micrometer
   1-mm graph paper
   Drying oven (optional)
   Sieves (500-μm mesh or smaller)

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Decomposition of Leaf Material

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I. INTRODUCTION

There are two primary sources of energy for streams: (1) instream photosynthesis by algae, mosses, and higher aquatic plants; and (2) imported organic matter from streamside vegetation (e.g., leaves and other parts of vegetation). In small, heavily shaded streams, there is normally insufficient light (see Chapter 5) to support substantial instream photosynthesis (see Chapters 17 and 28), thus energy pathways are supported largely by imported (allochthonous) organic matter. In such streams, the bulk of imported organic matter enters as dead leaves during autumnal leaf-fall, although greenfall (premature dropping of green leaves) may occur as fragments of fresh leaves due to herbivory by canopy arthropods or abiotic factors like wind (e.g., Risley and Crossley 1988). Additional dead leaf material may slide or blow into the stream from riparian zones over the rest of the year (Benfield 1997). In contrast, Gessner *et al.* (1999) reported that many riparian trees in central Europe normally shed fresh leaves and those leaves tend to maintain their structural integrity after being immersed in water.

Leaves falling into streams may be transported short distances but usually are caught by structures in the streambed and form "leaf packs" (Petersen and Cummins 1974; see also Chapters 13 and 31). Leaf packs are then "processed" in place by components of the stream community in a series of well-documented steps.

Dead leaves entering streams in autumn are nutrition-poor because trees resorb most of the soluble nutrients (e.g., sugars, amino acids, fatty acids) that were present in the green leaves (Suberkropp *et al.* 1976, Paul *et al.* 1978). Within one or two days of entering a stream, many of the remaining soluble nutrients leach out of the leaf's cellular matrix into the water, although there is evidence that some soluble materials remain in dead leaves long after they have been immersed in water (Paul *et al.* 1978). Furthermore, greenfall tends to retain soluble materials for some time after entering the water

(Gessner *et al.* 1999). After leaching, dead leaves are composed mostly of structural materials like cellulose and lignin, neither of which is very digestible by most animals. Within a few days of entering the water, fungi and bacteria begin to colonize the leaves leading to a process known as "microbial conditioning" (Bärlocher and Kendrick 1975). The microbes produce a suite of enzymes that can digest the remaining leaf constituents and begin the conversion of leaves to smaller particles (Suberkropp and Klug 1976). After about two weeks, leaves undergoing microbial conditioning begin to soften and some species may begin to fragment. Laboratory studies have shown that, given sufficient time, some species of aquatic hyphomycete fungi (see Chapter 15) can reduce whole leaves to small particles (Suberkropp and Klug 1980). However, reduction in particle size from whole leaves as coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM) is generally thought to occur through the feeding activities of a variety of aquatic invertebrates collectively known as "shredders" (Cummins 1974, Klug and Cummins 1979; see also Chapters 12, 13, and 25). Shredders help reduce the particle size of organic matter through the production of "orts" (i.e., fragments shredded from leaves but not ingested) and fecal pellets. The particles then serve as food for a variety of micro- and macroconsumers. FPOM may also come from a variety of other sources, both within and outside of streams (e.g., see Klug and Cummins 1979, Gessner *et al.* 1999). Leaves may also be fragmented by a combination of microbial activity and physical factors such as current and abrasion (Benfield *et al.* 1977, Paul *et al.* 1978).

Leaves from various tree and shrub species break down at different rates. Thus, there is a "leaf processing continuum" in most streams (Petersen and Cummins 1974) in which leaves from some species disappear rapidly ("fast processors"), some disappear moderately rapidly ("medium processors"), and some very slowly ("slow processors"). The consequence of this leaf processing continuum is that the stream community is supplied with leaf material as a food for much of the annual cycle (Petersen and Cummins 1974). Differences in the rates at which "fast," "medium," and "slow" species break down in a particular stream appears to be mostly a function of initial physical and chemical properties of leaves (Webster and Benfield 1986). Species-specific breakdown rates may vary with stream, location in the stream, time of year, activity of microbes, presence of shredders, and other stream-specific factors (Webster and Benfield 1986).

Leaf pack breakdown is an integrative, ecosystem-level process because it links various elements of stream systems (i.e., leaf species, microbial activity, invertebrates, and physical and chemical features of the stream). The major result of these linkages is that whole leaves are converted into fine particles which are then distributed downstream (see Chapter 13) and used as an energy source by various components of stream food webs (see Chapters 25 and 27). Clearly, leaf pack breakdown is not equivalent to leaf organic matter decomposition as pointed out by Boulton and Boon (1991), Gessner *et al.* (1999), and others. Decomposition is probably best defined as the conversion of organic matter into its inorganic constituents (i.e., mineralization). However, decomposition is certainly a part of the breakdown process and understanding leaf pack breakdown helps illuminate how energy flows through stream ecosystems (see also Chapter 31).

In recent years, leaf pack breakdown has been used to investigate long-term responses of streams to disturbance by logging (Benfield *et al.* 2001), responses of streams to acid mine drainage (Niyogi *et al.* 2001), influence of multiple land-use on stream structure and function (Sponseller and Benfield 2001), responses of streams to a gradient of agricultural development (Niyogi *et al.* 2003), and has been proposed as a useful method for assessing functional integrity of streams (Gessner and Chauvet 2002). The objective of this chapter is to provide a basic protocol for performing leaf pack breakdown experiments in streams

and to suggest some ways that leaf breakdown can be used to evaluate stream structure and function.

II. GENERAL DESIGN

The overall process of measuring leaf breakdown rates involves placing a group of preweighed "leaf packs" in a stream, periodically sampling from the group, and estimating the rate at which the packs disappear in the stream. Specifically, a large number of leaf packs are constructed and placed in a stream on Day 1 of the study. Three to five packs are retrieved regularly over the course of the study (perhaps 3–7 months), cleaned of debris and invertebrates, dried to constant mass, and weighed. Species-specific breakdown rates (k) are computed using an exponential decay model that assumes the rate of loss from the packs is a constant fraction of the amount of material remaining. Operationally, k is the negative slope of the line produced by a linear regression of the natural log of percent leaf material remaining plotted against time.

Site Selection

Leaf breakdown studies can be performed in virtually any size stream but small, shallow streams present fewer problems than do large ones, especially for a class experiment. Streams with gravel or cobble substrates are preferable to those with sandy bottoms because of the difficulty of anchorage and the likelihood of burial in sandy-bottomed streams. Remote sites are preferable to sites that receive regular human traffic because leaf packs are attractive to the curious. Avoid spots that are likely to be significantly deeper during higher flows, areas of excessive erosion (e.g., next to cut banks) or deposition (e.g., point bars), and areas that may be unstable under higher flows (e.g., debris dams). Best results are usually obtained when leaf packs are placed in shallow riffles closer to the bank than the middle to avoid increased stream power during high flows.

III. SPECIFIC METHODS

A. General Protocol

1. Collect leaves from trees just before they are ready to fall (i.e., at abscission) or shortly after they fall but before they are exposed to rain.
2. Air dry the leaves by putting them into large cardboard boxes with many holes (about 3 cm dia) covered by plastic window screen and placing the boxes in a dry location. Invert the boxes daily and gently "fluff" the leaves to promote drying. Continue 5–8 days until leaves reach relatively constant dry-mass. Alternatively, leaves can be spread out on the floor or tables to dry. Thick piles of leaves should be turned over frequently to promote drying.
3. Weigh out 3 to 10 g (± 0.1 g) portions on a top-loading analytical balance, and fashion them into leaf packs by one of several techniques described below. It is advisable to use the same mass (weight) of leaves for each pack. Record the initial dry mass on a data sheet (see Table 30.1).
4. Construct mesh bags from bridal netting or similar material such as poultry fencing, hardware cloth, or large mesh plastic screen. Commercial mesh bags used

TABLE 30.1	Example Data Sheet for Leaf Breakdown Study (All Values in Grams).

Sample ID	LPDM	PM	P+DM	P+AM	DM	AM	%Organic	AFDM
MAPLE 1*	8.27	1.0000	1.2500	1.0250	0.2500	0.0250	90	7.44
MAPLE 2	_____	_____	_____	_____	_____	_____	—	___
MAPLE 3	_____	_____	_____	_____	_____	_____	—	___
OAK 1	_____	_____	_____	_____	_____	_____	—	___
OAK 2	_____	_____	_____	_____	_____	_____	—	___
OAK 3	_____	_____	_____	_____	_____	_____	—	___
Days of incubation: _____								

*Example data
Note: LPDM = dry mass of leaf packs; PM = pan mass; P+DM = pan mass plus pre-ashed mass of milled sample; P+AM = pan mass plus post-ashed mass of milled sample DM P + DM − PM); AM = (P+AM − PM); %Organic = (DM − AM/DM × 100); AFDM = (DM × %Organic)

to package produce (e.g., nonwicketed Grape Bags in lots of 1000 from nswplastics.com) also work well. Regardless of the material used, mesh openings should be large enough to allow access to consumers yet small enough to retain the leaf material (Webster and Benfield 1986).

5. Prepare enough packs for the entire exposure period. Variability in the amount of material lost from leaf packs is relatively high, especially in the later stages of decomposition. Thus, a minimum of three packs per species per site should be retrieved on each date in order to calculate a mean and standard error. In experiments involving more than one species, select a method to differentiate the leaf packs by species because it can be difficult to distinguish between species when the leaves are in the middle to later stages of decay. Color coded plastic "embossing" tape stapled to the bags works well, as do bags of different colors or strips of "flagging tape" tied to the bags.

6. By their very nature, dried leaves are easily broken in handling. Therefore, it is necessary to account for losses encountered in fashioning, transporting, and placing the packs in the stream. This can be accomplished by preparing an extra set of leaf packs that goes through the entire process but are not left in the stream to incubate. The extra packs are processed and used to correct for "handling losses" (described following).

7. Set up a retrieval schedule according to the leaf type used. "Fast" leaf species may disappear in 1–3 months and packs should be collected weekly or every two weeks. "Medium" and "slow" leaves may require 4 to 12 months to disappear and may be collected at monthly intervals (Webster and Benfield 1986).

8. Transport all packs to the stream site, handling carefully to avoid unnecessary breakage.

9. Packs must be secured in the streambed. Depending on size and flow rate of the stream, various restraint systems are recommended. In small, shallow streams, leaf packs in mesh bags can be tied with polypropylene twine singly or in groups to "gutter" nails (9" nails used to attach guttering to houses) pushed or driven into the streambed (Webster and Waide 1982). In larger or faster flowing streams, steel rods or metal fence posts driven into the streambed may be necessary to anchor the leaf packs. Alternatively, attach the packs with "zip-ties" to heavy wire tied to a tree along one bank (Benfield et al. 2000). If none of these techniques seem

appropriate, stronger devices such as those described in Benfield and Webster (1985) may be necessary.

10. Place all packs, including those designated for "handling loss" correction, in the stream spreading them out as much as possible as governed by the restraint system chosen, available space, etc. If more than one leaf species is used, each species should be spread randomly through the array. After the leaf packs are in place and secured, retrieve the packs designated for handling loss correction, while leaving the other packs for the experiment. Drawing a simple map showing the location of the leaf packs may be helpful in locating the packs when it is time to retrieve them.

11. Following the retrieval schedule, remove the appropriate number of leaf packs and place each pack into individual Ziploc®. Include an internal label (pencil or permanent marker on waterproof paper) identifying the sample with all pertinent information (e.g., retrieval date, site, species, etc.). Write the same information on the outside of the Ziploc®, using a permanent marker. Place the samples on ice in a cooler and return them to the laboratory. Keep the bags in the cooler or refrigerate until processed.

12. Processing in the laboratory may involve several options depending on whether you decide to measure only leaf pack breakdown or breakdown plus additional work with macroinvertebrates (see Chapters 20, 21, and 25), microbes (see Chapters 14 and 15), leaf chemistry or stable isotopes (see Chapter 27), or other components. In any case, remove the leaf material and gently rinse the leaves of silt and debris. Because the breakdown model represents loss from the original mass, ignore small leaf fragments that may have been lost from the original mass but retained by the bag. Place the cleaned leaves in paper bags. Keep the field labels with the individual packs as you transfer them into the paper bags. Label the outside of the paper bags with the same information that appears on the inside labels. Hang the bags on a line stretched across the laboratory and allow the leaf material to air dry to constant mass. Alternatively, dry in a hot air oven at 50°C or less for at least 24 hr. After drying, weigh leaf material and record the dry mass (DM) on the data sheet. If the project calls for saving macroinvertebrates, perform the rinsing over a 250 μm sieve and place the macroinvertebrates in 70% ethanol with appropriate labeling inside and outside the containers (see Chapter 20). If you plan to do microbial analyses, subsample the leaves before drying and keep the subsamples cold and moist or frozen depending on the protocol required for the particular analyses planned (e.g., see Chapters 14 and 15).

13. In many cases, mineral deposits are not readily washed off the leaves and may result in errors in final dry mass. This problem can be mostly overcome by converting dry mass to ash-free dry mass (AFDM). Organic matter combusts at about 550°C and the remaining material is mineral ash. When the mass of mineral ash is subtracted from initial dry mass, the result is the dry mass of the "organic fraction" (ash-free dry mass or AFDM) of the leaf material. For each species, process the leaves by milling (by Wiley Mill) or grinding (by mortar and pestle) all or significant portions of the "handling loss" leaves and the leaves from each retrieval (all packs combined for each date) to a fine powder. Determine AFDM for the leaf packs as described below in #14. The AFDM of the "handling loss" leaves serves as the initial AFDM (i.e., the AFDM of the leaves before they were put into the stream).

14. Mark the underside of aluminum weighing pans by inverting them over the bottom of a beaker and impressing a code using a metal probe. Record the

identification codes on the data sheet. To obtain the tare weight of each pan, heat the coded pans at 550°C for 30 minutes in a muffle furnace. Then, handling with gloves and tongs or forceps as appropriate **throughout the process**, place the pans in desiccators to cool. After cooling, weigh and record the mass (weight) of each pan at the appropriate place on the data sheet. Weigh out at least 2 subsamples of about 250 mg DM of each milled sample into a tared pan, oven-dry over night at 50°C, and place in a desiccator to cool. Weigh the pans plus milled samples and record on the data sheet. Place pans plus milled samples in a muffle furnace at 550°C for 20 min, remove and stir with a dissecting needle, then return pans and sample to the furnace and heat for an additional 20 min (Gurtz *et al.* 1980). Remove pans from the furnace and allow to cool in a desiccator. After cooling, wet down material with distilled water, then oven dry at 50°C for 24 hr. Remove pans with samples from the drying oven and desiccate. After cooling in the desiccator, weigh and record pan plus ash on the data sheet. Subtract the pan weights from the preashed pan plus sample and post-ash pan plus sample. Compute % organic matter of the milled samples as follows:

$$\% \text{ organic matter} = [(\text{sample dry mass} - \text{sample ash mass})/ \atop (\text{sample dry mass})] \times 100 \ \% \qquad (30.1)$$

15. Convert DM values of leaf packs to AFDM:

$$\text{AFDM} = (\text{DM}) \times (\% \text{ organic matter}) \qquad (30.2)$$

16. Convert AFDM for each leaf pack to % AFDM remaining:

$$\% \text{ AFDM remaining} = \text{Final AFDM/Initial AFDM} \times 100 \qquad (30.3)$$

17. Regress the natural log (ln) of mean % AFDM remaining (y-axis) on days of exposure (x-axis) using the AFDM of the "handling loss" leaf packs as 100% remaining for Day 0. The negative slope of the regression line is equal to the processing coefficient (k).

B. Basic Method 1: Leaf Breakdown for One or Several Leaf Species

Perform a leaf breakdown study using one or several leaf species at one site in a stream. Install a water temperature monitoring device at the site. Compute the processing coefficient (k) for AFDM loss and for cumulative temperature (degree-days). Processing

coefficients (k) can be computed using cumulative temperature (degree-days) as values on the *x*-axis in place of days (Petersen and Cummins 1974). Cumulative degree-days may be estimated by summing the average daily water temperature over each incubation period and entering the appropriate values (i.e., the degree-days accumulated from day 1 to the retrieval day) in place of days.

The simple single-species-single site in one stream model can easily be expanded by including more species. For example, one could contrast the breakdown rates of presumed "fast," "moderate," and "slow" species. Another model could be to contrast riparian shrub or herbaceous leaves with tree leaves, or perhaps deciduous and evergreen shrub and/or tree leaves. Processing coefficients (k) may be compared statistically using analysis of covariance (ANCOVA) or a Dummy Variable Regression (DVR) to determine whether the k values are significantly different (see Kleinbaum *et al.* 1988, Sokal and Rohlf 1995, or Zar 1999).

C. Basic Method 2: Effects of Spatially Varying Stream Features on Leaf Breakdown Rates

Many designs are possible depending on the question(s) of interest. For example, use one or several species of leaves at single or multiple sites in one or several streams. Site differences could include riffles versus pools, high elevation versus low elevation, cobble substrate versus bedrock or sand, or shaded versus unshaded reaches. Stream differences could be based on stream order, gradient, geology, disturbance history, hardness, or nutrient level. In sand bottom streams, leaves often become buried by transported bed material. The experiment may include comparing breakdown rates of buried vs. unburied leaves (e.g., Tillman *et al.* 2003). Comparisons of site-species combinations or treatments can be analyzed in a manner similar to the statistical methods described in the General Protocol.

D. Advanced Method 1: Effects of Anthropogenic Activities on Leaf Breakdown Rates

Investigate the impact of a municipal, industrial, or mining waste outfall on stream organic matter processes using leaf breakdown rate as an indicator (e.g., Paul *et al.* 1983, Nyiogi *et al.* 2001). The usual protocol for evaluating the impact of a waste outfall on streams is to compare some value(s) upstream and downstream of an outfall at comparable sites (for a general discussion see Plafkin *et al.* 1989). Establish an upstream "reference" site(s) that is totally removed from any possible impact of the outfall in question. In wider streams, you may also use sites across the stream as reference sites. Establish a site just downstream from the outfall where impact is likely to be maximal, and then several additional sites further downstream including one or more at which you judge the impact of the outfall to be abated. Proceed with the study as outlined above in the "General Protocol" and analyze for site differences as described in Basic Method l.

E. Advanced Method 2: Assessing Relationships Among Leaf Breakdown Rates, Shredders, and Microbes

Exploring the relationship of shredders and microbes to the breakdown rates of leaves in any of the protocols outlined above can be accomplished by planning ahead as described in "General Protocol" Step 12 above. Macroinvertebrates sorted from the leaf packs can

be identified and placed into functional feeding groups as described in Chapter 25. Regression analysis can then be used to evaluate relationships between macroinvertebrate number per bag, density, or biomass and breakdown rates where there are sufficient data (e.g., Sponseller and Benfield 2001, Niyogi *et al.* 2001). A word of caution: Macroinvertebrate numbers in litter bags can be extremely variable, especially toward the end of the breakdown process. Microbial activity on organic matter undergoing breakdown is an important indicator of the decomposition process that is key to the reduction of CPOM to FPOM. Microbial activity can be followed by estimating accumulating microbial biomass by measuring ergosterol, the major sterol in the membranes of higher fungi. Fungal production can also be estimated by measuring the rate of radio-labeled acetate into ergosterol (see Chapter 15). Finally, microbial respiration on decaying leaves can be measured by following changes in oxygen uptake by microbial communities on leaf material (Niyogi *et al.* 2001 and see Chapter 14).

IV. QUESTIONS

1. Are leaf pack breakdown and organic material decay essentially the same process? Why or why not?
2. What might be the impact to energy flow in a woodland stream if streamside (riparian) vegetation composition were simplified by removing all but one or two species? Can you think of examples where this has been done?
3. By what mechanisms might a "pollution" source alter the process of leaf breakdown in streams?
4. How might you attempt to experimentally separate the importance of biological process (i.e., microbial conditioning and consumer feeding) from physical processes such as abrasion and fragmentation by currents in breaking down leaves in streams?
5. What are some of the variables that make some leaves more resistant or susceptible to leaf breakdown processes in streams?

V. MATERIALS AND SUPPLIES

Equipment

Top loading analytical balance accurate to 0.1 g
Conventional analytical balance accurate to 0.1 mg
Muffle furnace
Forced air drying oven
250-μm mesh sieves
Grinding mill (or mortar and pestle)

Supplies

Mesh bags
Gutter nails
Steel rebar
1/8 inch cable — as needed depending on leaf pack design
Ziploc® bags
Paper bags
Labels

Tape
Waterproof marker
Aluminum weighing pans
Tongs
Gloves
Forceps

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Riparian Processes and Interactions

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I. INTRODUCTION

The streamside (riparian) environment adjacent to the open channel is the principal interface between the land and streams. Because of the integral relationship that exists between stream and riparian environments, the two often are regarded as constituting a single ecosystem (Minshall 1988, Cummins *et al.* 1989, Gregory *et al.* 1991). The riparian zone encompasses the streambank and floodplain vegetation, as well as any vegetation outside the floodplain that is likely to enter the stream by gravity (*recruitable debris*) (Minshall 1994). This latter aspect is especially important in streams located in steep-sided valleys and in forests of tall trees.

The riparian environment forms a transition zone between the open stream and the adjacent uplands. The stream/riparian interface may be a sharp boundary (edge) or a gradual transition (ecotone) between the two. The size and distinctiveness of the riparian border and whether it is viewed as a distinct boundary or an ecotone depend on the harshness of the environmental conditions encountered between open water and uplands. The sharpness of the environmental gradient is a function of a number of factors including climate, topography, land form, and geological control (constraint) (Gregory *et al.* 1991). Shallow groundwaters, interconnecting with the *riparius* (noun-form of the adjective *riparian*), supply dissolved nutrients to the open stream channel via the hyporheos through microbial transformations and transport and can locally enhance stream productivity (Valett *et al.* 1994). The hyporheos is a region of shallow groundwater (Figure 31.1), which is intermediary between and interconnected to the surface water of the open stream channel and the deeper groundwater (Boulton *et al.* 1998). Laterally,
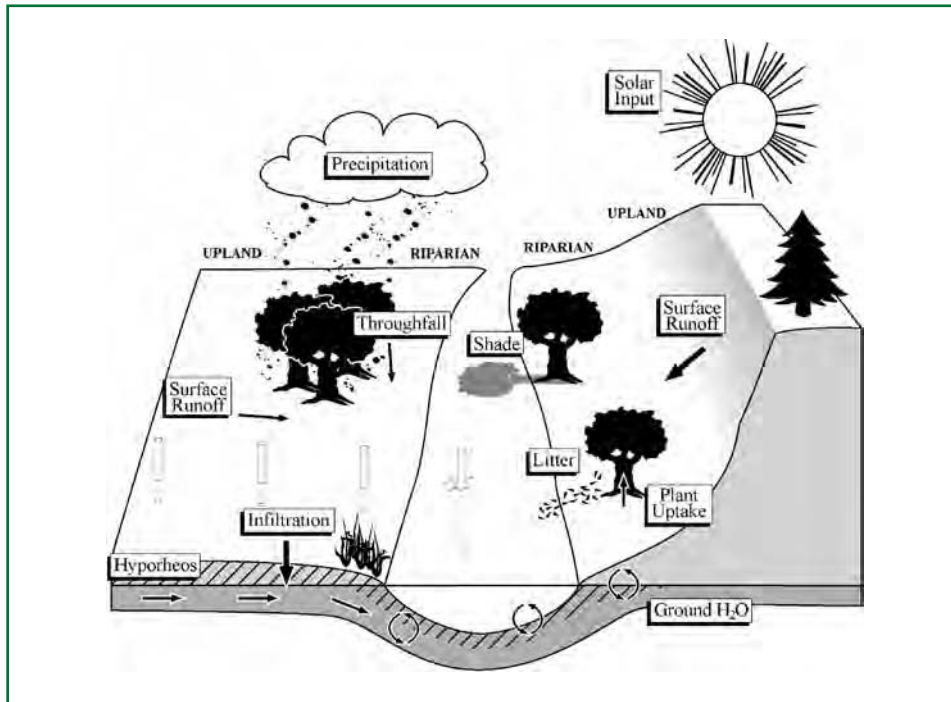
the hyporheos extends into the riparian region where it influences and is influenced by the riparian vegetation. Temporally, the interchanges are most active during periods of high hydraulic head, usually associated with extensive surface water runoff from the land. The riparius may or may not include a floodplain. In rivers with extensive floodplains, annual floods result in lateral interactions between the open channel and the riparius. These interactions may be surficial, as explained by the flood pulse concept (Junk *et al.* 1989, Bayley 1995) and/or may have a strong subsurface component associated with the hyporheic zone (Stanford and Ward 1993, Brunke and Gonser 1997; Chapters 6 and 33).

Riparian habitats are especially important as refuges during periods of environmental stress, such as annual drought or rapid shifts in climate, because of the ameliorated climates they provide along river valleys (Gregory *et al.* 1991, Minshall 1992). The riparian environment strongly influences the microclimate, physical structure, and food resources of the open stream (Gregory *et al.* 1991). This influence is driven largely by the makeup and density of the vegetation, which in turn is strongly influenced by soil, water, temperature, and light conditions. Terrestrial leaf litter constitutes an important food resource for lotic consumers. Terrestrial woody debris provides physical habitat, modifies streamflow and channel conditions, and retains organic matter of smaller sizes. The influence of riparian vegetation, the annual amount of terrestrial leaf litter in the channel, the availability of dissolved organic matter, and the modal size of particulate organic matter all vary with the distance from the headwaters of a stream system (Vannote *et al.* 1980).

In the riparian environment, several factors are critical that also are important in the open stream channel (e.g., light, temperature, nutrients). But several additional factors also are important, including: soil type and depth, moisture availability (e.g., proximity to the water and extent, frequency, duration of flooding), width of the riparian zone, and bank stability (Minshall *et al.* 1989, Gregory *et al.* 1991). The principal biotic components of riparian habitats are comparable to those of the open water in streams but the primary producers are predominantly woody terrestrial plants (especially shrubs), sedges, and grasses instead of algae and flaccid vascular plants, and the vertebrate consumers are mainly birds and mammals, in place of fish. Key features used in describing the riparian plant community include the age composition, structural composition, cover, and overhang of terrestrial vegetation and the amount, size, and distribution of woody debris (e.g., Platts *et al.* 1989, Burton *et al.* 1991, Cowley 1992).

Streamside vegetation is a major source of energy and nutrients for instream communities. However, reverse flows of carbon from aquatic to riparian ecosystems occurs through predation by terrestrial mammals, birds, or invertebrates on fish and aquatic insects (Collier *et al.* 2002). Coarse particulate organic matter (CPOM), which enters streams from the adjacent land as leaves, twigs, seeds, and other forms, plays an especially important role in the trophic dynamics of flowing (lotic) waters (Minshall 1967, Vannote *et al.* 1980, Cummins *et al.* 1989). The adjacent riparian vegetation largely determines the extent to which the surface of a stream is shaded. Availability of light regulates the occurrence and growth of algae and higher aquatic plants. Shading also moderates the thermal regime of stream communities by providing cooler temperatures, which benefit most aquatic life (Swanson *et al.* 1982). Removal of riparian vegetation can result in increases of water temperature and, consequently, alterations in levels of dissolved oxygen, invertebrates, and fish. Riparian areas perform a number of important functions including (1) physical filtration of water, such as removal of sediment (Cooper *et al.* 1987) and heavy metals; (2) bank stabilization; (3) water storage and recharge of subsurface aquifers; (4) nutrient retention, transformation, and release (Lowrance *et al.* 1984a, 1984b, Cooper and Gilliam 1987, Green and Kaufman 1989, Triska *et al.* 1989); (5) regulation of light and thermal conditions in streams (Hill and Dimick 2002, Kiffney *et al.* 2004); (6) provision of organic matter to aquatic consumers (Minshall 1967, Cummins *et al.* 1989); (7) generation of food web changes (allochthonous vs autochthonous) (Tabacchi *et al.* 1998, England and Rosemond 2004); and (8) provision of corridors for the dispersal of plants and animals (Gregory *et al.* 1991). However, most of these processes presently lack a standardized assessment methodology and/or are not routinely measured. One exception is the measurement of transient storage, which relates to items 3 and 4, and is increasingly being assessed (see Chapter 8).

Below we present a suite of methods for assessing riparian processes and interactions at the scales of stream reaches or segments. These measurements may be scaled up through assessment at multiple locations and the use of remote sensing (Cummins *et al.* 1989) and Geographical Information Systems. At the scales of watersheds and larger, additional GIS layers such as topography, vegetative cover, road density, and so forth may be included (Chapter 1).

II. GENERAL DESIGN

As just noted, there are a number of structural and functional attributes of importance in riparian habitats. Riparian zones often are distinguished on the basis of hydrology,

vegetation, and soils (Swanson *et al.* 1982, Lowrance *et al.* 1985, Tabacchi *et al.* 1998). From a functional perspective the riparian zone is an area of direct interaction between terrestrial and aquatic systems involving exchanges of energy and matter (Gregory *et al.* 1991). The focus of this chapter will be the assessment of the attenuation of solar radiation through shading; the input, transfer, and processing of coarse organic matter; and the transfer of dissolved organic matter and nutrients.

A. Attenuation of Solar Radiation/Shading

Solar radiation affects primary production and the thermal environment in streams. The focus of interest could be either the surface of the stream or of the soil within the riparius. The extent of shading by riparian vegetation can be evaluated as (1) the degree (absolute or relative percent) to which solar radiation is diminished relative to that received on unobstructed bare ground and (2) the spatial extent and duration of a shadow cast by overhead vegetation. In both of these approaches, care must be taken to avoid or correct for shading by mountains, canyon walls, buildings, or other obstructions.

In the first instance, incoming solar radiation is measured at the water or soil surface with a suitable pyranometer or quantum sensor (Chapter 5) at multiple locations along a stream segment and compared with a set of values obtained from an unshaded sensor. An integrated value for the day or an instantaneous value obtained at some standardized time, usually midday, may be used either individually or in combination. Because of cost constraints, the integrated measure is more suitable for a single, fixed site and the instantaneous measure is more applicable for multiple locations. Depending on the instrumentation, integrated values may be made continuously or estimated from hourly values (at least 0900, 1200, 1500, and 1800 h). Solar radiation values will change seasonally with shifts in the angle of the sun and as a function of cloud cover, leaf development, and dominant plant species and age so these factors would need to be considered in a more comprehensive investigation.

The second approach to the determination of shading, which also measures solar insolation directly and in terms of energy units, is to use a Solar Pathfinder instrument (www.solarpathfinder.com) which estimates energy input based on location and the portion of total available energy actually reaching a site (Platts *et al.* 1987, Davis *et al.* 2001). The instrument is set up in the middle of the stream; obstructions that would block solar input are reflected on its domed surface. The reflection is then outlined on a solar chart and values representing the percent of total daily input are determined. The instrument usually is used to determine total annual solar energy input but, with appropriate modifications, daily values can be obtained. Results are most accurate when only a few trees or shrubs are present and are of limited use with a dense riparian canopy. An alternative procedure is to determine shading indirectly by measuring canopy density using a spherical densiometer. Canopy density is a measurement of the amount of overstory vegetation that prevents direct sunlight from reaching the stream surface with measurements taken at both banks and in the middle of the stream. For each transect, every measurement represents 1/4 of the total density and the sum of the individual measurements is multiplied by 1.5 (Platts *et al.* 1987) to determine the canopy closure or density for that transect (Platts and Nelson 1989). By expressing the results relative to readings from a totally unshaded area, measurement may be obtained in terms of "% shaded."

Quantifying solar energy input to streams allows subsequent examination of factors that may vary with this input. These include interactions between light and algal

production (Kiffney *et al.* 2004) and the subsequent effects on invertebrate grazers. For example, the gain of leaves by riparian plants in the spring and their loss in the autumn, through their alteration of streambed light regimes, can affect periphyton photosynthetic characteristics and thus primary production in streams (Hill and Dimick 2002). Being able to measure riparian canopy and light energy also leads to a better understanding of the effects that anthropogenic disturbances may have on aquatic systems. Effects of shading on the thermal environment can be assessed by measuring the temperature of the air, water, and soil simultaneously with that of the solar radiation measurements (Chapter 5).

B. Input and Decomposition of Coarse Organic Matter

Much of the organic matter in a stream may originate from the surrounding terrestrial environment and be transported to the stream by wind, water, gravity, or direct deposition. Because of its origin outside of the stream boundaries, this material is referred to as *allochthonous* and is primarily of plant origin. Since much of this allochthonous plant litter, in the form of leaves, twigs, and other parts, is dead by the time it reaches the stream, it also is often referred to as *detritus* (i.e., allochthonous detritus). In many cases, particularly in shallow (wadeable) streams in forest or shrublands, organic matter of terrestrial origin plays a major, often overriding, role in establishing stream ecosystem structure and function (e.g., Fisher and Likens 1973, Minshall *et al.* 1983, Wallace *et al.* 1997). Valuable insights into the dynamics of flowing water ecosystems and terrestrial-aquatic linkages are based on the changing terrestrial dependence of these systems with different biogeographical areas, increasing channel size, varying types and amounts of streamside vegetation, different land-use practices, and the dynamics of input, storage, processing, and output of organic matter (e.g., De La Cruz and Post 1977, Cummins *et al.* 1983, Minshall *et al.* 1983, 1992, Duncan and Brusven 1985a, 1985b, Naiman *et al.* 1987, Duncan *et al.* 1989, Meyer 1990).

Accurate determination of leaf input and other forms of organic matter contributions to streams and soils by riparian plants is a daunting task. In the past, the most common approach has been to place containers, such as plastic laundry baskets, over open water or along the stream banks in an attempt to estimate these litter (allochthonous detritus) inputs. But such methods have suffered from a number of problems including nonrandom placement and/or inadequate sample size. In addition, they only allow estimation of the portion that falls directly into the stream or on the ground and miss any lateral movement on land by gravity, wind, or other means. This lateral movement generally enhances inputs to streams but may either add to or subtract from deposits on the ground. One promising approach is to collect all of the litter deposited on netting of known area spread over the stream at bank level (for stream input by all vectors exclusive of upstream transport) and/or on the ground (for riparian input). Some have attempted to estimate litter inputs by measuring its occurrence in the benthic organic matter component of streams (see Chapter 13), but the values obtained are affected to a variable and unknown extent by stream transport into and out of the location from which the collection is made and by the clumped distribution of leaves in streams. Very few studies have attempted to measure or separate the individual components of litter input (direct fall in, lateral transport, etc.) and we know of no study that has measured litter production for an entire riparian zone or determined its relative contribution to the ground surface and adjacent stream. One approach, though labor intensive, is to estimate litter production on a plant-volume basis for each of the main forms of riparian plants, scale that up to the total volume

of each of those plants within the area of the riparian zone of interest, and determine direct fall-in and lateral inputs to the stream as indicated above. A simplification of this method, though less comprehensive and informative, is to use the line intercept method to determine riparian plant species composition and relative abundance and relate this to the amount of leaf and needle litter that becomes trapped in the adjacent stream reach (Cummins *et al.* 1989). Another approach is to determine the litter production for the vegetation within the riparian zone and then, by use of a suitable tag such as a stable isotope, measure the portion that ends up in the stream. Both of these two main methods are described in this chapter.

Litter input into the riparian and stream channel locations is a direct function of plant cover (density and canopy volume) and valley side slope. Plant cover will vary with soil moisture and a gradient generally extends from the highest moisture levels near the edge of the stream channel to the lowest values near the outermost edge of the riparian zone. Thus, a similar gradient in riparian plant density and canopy volume also usually is present. In addition, input from the riparius to the channel will decrease logarithmically with distance from the stream as a result of decreases in litter availability and ease of recruitment. Thus, advanced sampling designs should incorporate this aspect of spatial variation.

Litter decomposition and linkages to communities of stream invertebrates are strongly related to plant type (Cummins *et al.* 1989), so it is important to characterize the allochthonous detritus production of plant species according to the particular processing category to which they belong. Leaves are generally recognized as belonging to one of three litter processing categories in terms of rates of decay: **fast** (>0.15% dry weight loss per day normalized for temperature), **medium** (0.10–0.15%), and **slow** (<0.01%). Common representatives of each category are: Fast — *Alnus, Cornus, Fraxinus, Liriodendron, Prunus*; Medium — *Acer, Populus, Salix, Ulmus*; Slow — *Quercus, Rhododendron, Pinus, Platanus, Tsuga* (Peterson and Cummins 1974, Webster and Benfield 1986, Cummins *et al.* 1989). Leaf quality in terms of tannins (−), N (+), C:N (−), and lignin (−) are significantly correlated with processing rates (Ostrofsky 1997).

Decomposition processes represent a major flux of both fixed carbon and nutrients in most terrestrial systems, and quantifying rates of litter mass loss or respiration and the concomitant changes in nutrients bound in the litter are important aspects of evaluating ecosystem function. Plant litter decomposition (see Chapter 30) plays an important role in determining carbon and nutrient accumulation in riparian soils, as well as the rate and timing of nutrient release in forms available for uptake by plants and soil biota. Like in-stream decomposition of terrestrial litter, litter decomposition or preconditioning on riparian soil is controlled to varying degrees by abiotic and biotic conditions (Wagener 1998). The decomposition process transforms senescent plant material into both labile and stable organic matter both above and belowground (Harmon *et al.* 1999). The dynamics of riparian decomposition are such that nutrients in decomposing litter can act as either a nutrient sink (nutrients retained in riparian area) or a source (nutrients transported to the stream) relative to adjacent streams. Standing stocks of litter represent important carbon and nutrient reservoirs. The sizes of these reservoirs are influenced by both rates of litter production and decomposition and are sensitive to changes in either process.

Methods for measuring riparian litter decomposition are less problematic than those for litter input and generally involve measurement of loss of biomass over time from leaves in exposed packs or contained within mesh bags (Chapter 30). This approach is equally applicable to stream or riparian habitats. However, leaf processing is scale-dependent

and factors controlling processing rates will differ depending on the spatial scale of study (Royer and Minshall 2003). Because of differences in decay rates, it is advisable to keep leaves of individual species separate when measuring leaf decomposition. However, use of different kinds of leaves in separate accumulations representing more than one decay-rate category can provide added insight because the particular mix of leaf species may affect natural leaf pack decomposition rates (MacArthur *et al.* 1994, Kaneko and Salamanca 1999, Swan and Palmer 2004).

On riparian soils, the litter input provides a direct source of food to invertebrate consumers, such as earthworms; serves as a substrate for microbes; and, through leaching and decomposition, releases nutrients needed by plants. In streams, this "allochthonous detritus" serves a similar function and is especially tightly linked to characteristic fungi and the shredder functional feeding group of macroinvertebrates. Therefore, in addition to measuring the rate of leaf decay, the strength of these linkages on riparian soils and in streams can be determined through assessment of fungal and detritivore standing crops. On riparian soils, additional indicators include terrestrial plant growth rates and size, and soil nutrient concentrations.

C. Transfer of Dissolved Organic Matter and Nutrients from the Riparius to the Stream

Riparian zones control energy and material flow to streams (Naiman and Decamps 1997) through interacting, simultaneous processes, and riparian zones can serve both as sources or sinks for energy and matter. In riparian zones, biogeochemical processes that affect streamside as well as in-stream ecosystems occur at multiple scales and vary depending on the type of vegetation (e.g., N-fixing, desert shrub, grassland, and coniferous). Riparian vegetation, organic matter supply, and decomposition are important components (section IIB) responsible for nutrient dynamics within the riparian soil. Dissolved organic carbon (DOC) and nutrients are transported into, through, and out of riparian habitats primarily by precipitation, surface runoff, and groundwater (Figure 31.1). Water chemistry is altered as it passes through the riparian zone along hydrologic pathways from uplands to streams. These zones function as control points for fluxes of nitrogen (N) and other nutrients from terrestrial to aquatic systems (Hedin *et al.* 1998). For example, Mulholland (1992) found higher concentrations of soluble reactive phosphorus (SRP) and inorganic N in riparian groundwater and springs than in upslope soil solution or stream water, suggesting within-watershed sources of these forms and additional removal by in-stream processes. DOC and nutrient inputs to riparian zones can be measured through collection and analysis of precipitation, surface runoff from uplands, and groundwater to investigate transformations occurring as water moves through the riparian zone. These values may differ depending on time of collection relative to hydrologic conditions, whether during baseflow, rainstorms, or snowmelt during spring runoff. In this section we examine the processes and interactions in the riparian zone involving exchanges of energy and matter, resulting in the regulation of the movement of materials in the soil and groundwater, and the effects of these processes on food web structure in streams.

Precipitation and canopy leaching (via throughfall) can potentially be large sources of important nutrients, such as N and phosphorus (P), to the stream, whereas soils can be a major sink (Mulholland 1992). Riparian vegetation removes and retains particulates, which favors soil microbiological processes and increases soil nutrient cycling, thus reducing nutrient inputs to streams. In most forested watersheds, biological and geochemical processes in upper soil horizons effectively retain N and P, thus reducing inputs to

streams (Wood *et al.* 1984). Soil texture also plays an important role in determining relative proportions of surface water and groundwater inputs. Processes that occur in the soil are influenced by redox (reduction or oxidation) conditions. For example, increasing oxidation of soils leads to increased nitrate concentrations due to nitrification whereas reduction leads to increased ammonium concentrations through denitrification (see Chapter 10). Reduction processes require that soils be anaerobic or of low redox potential (Eh) and oxidation processes require the opposite conditions. Low Eh is a result of belowground processes consisting of biogeochemical reactions that transfer electrons from organic matter released from plants, to various terminal electron acceptors (Tabacchi *et al.* 1998). These changes in redox conditions can be measured through the analysis of water samples taken from the stream, groundwater, and upland areas.

The main control on the interaction of groundwater with stream riparian zones is the hydrogeologic setting, which encompasses surface topography, soils, and the composition, stratigraphy, and hydraulic characteristics of the underlying geological deposits. To examine the role of groundwater in riparian-stream interactions, transects are established for groundwater wells (piezometers) and lateral wells on either side of the groundwater well in the riparian zone (Figure 31.1). Individual wells or clusters of wells may be used depending on the scale of study and the questions being addressed (Chapter 6). Water samples collected from the wells can be analyzed for nitrate, nitrite, total Kjeldahl-N, ammonium-N, total-P, orthophosphate-P, and organic matter concentrations. Redox or dissolved oxygen measurements within the well and stream water give insight into potential processes and reactions.

Stable isotopes may be used to further understand stream-riparian interactions and processes and address complex questions dealing with trophic interactions. Stable isotope analysis can be conducted for C and N in each of the main stages that may be encountered as a nutrient makes its way across the riparius and into the stream (e.g., abscised leaves, leaves conditioned in the stream and on the forest floor, periphyton, soil, groundwater, surface water, and invertebrates). This information can be used to determine pathways, compare riparian-zone processes in different ecoregions, and determine the effect of alterations, such as deforestation and agricultural practices, on riparian-stream processes. Stable isotopes also can serve as tracers of energy flow within food webs (Peterson and Fry 1987) and can be useful in establishing the relative importance of terrestrial versus aquatic energy sources (Finlay 2001). Stable isotopes of C and N can be used to discriminate between allochthonous and autochthonous pathways in food webs at specific sites (Rounick and Winterbourn 1986). This is dependent on the degree of isotopic differentiation between these two food resources (Chapter 27). The ratio of C isotopes changes little as carbon moves through food webs and, therefore, typically can be used to evaluate the ultimate sources of carbon for an organism when the isotopic signature of the sources are different (Collier *et al.* 2002). $\delta^{13}C$ and $\delta^{15}N$ signatures for in-stream algae, terrestrial leaves, and stream invertebrates may be used to distinguish aquatic versus terrestrial energy sources to consumers (Finlay 2001, England and Rosemond 2004). The extent to which groundwater decreases in nitrate in riparian sites is due to denitrification and/or plant uptake and is determined through measurements of nitrogen isotopes in both groundwater nitrate and riparian plant tissues (Clement *et al.* 2003).

Another advantage in using stable isotopes is to distinguish between marine and terrestrial sources of nitrogen. Isotopic ratios of $^{15}N:^{14}N$ are generally higher in marine systems and elevated $^{15}N:^{14}N$ in terrestrial systems are indicative of marine enrichment. The last decade has seen an increase in research examining N derived from marine sources (MDN), (Cederholm *et al.* 1989, Kline *et al.* 1993, Wipfli and Caouette 1998,

Thomas *et al.* 2003) and riparian areas dominated by alder (Binkley *et al.* 1985, 1992) to examine the effects on nutrient cycling and riparian and stream productivity. Riparian plants adjacent to spawning streams may derive up to 24% of foliar N from salmon (Bilby *et al.* 1996) and growth rates may be enhanced (Helfield and Naiman 2001).

D. Site Selection

The procedures described here lend themselves most readily to first- through third-order streams, but may be extended to up to sixth-order wadeable streams with a little ingenuity and without too much additional difficulty. In unconstrained valleys, the riparian zone consists of the strip of land between the stream channel and the hillslope whereas in narrow, constrained valleys the width extends to a distance equivalent to the height of the tallest trees growing on the hillslope. Choose a 250-m to 1-km long segment or segments of stream for study and determine the area of the riparian zone as the product of the segment length × the mean width of the riparian zone (from the outside edge on one side to the same point on the other side) determined from five or more transects oriented perpendicular to the channel. Generally the segment will contain a reasonable degree of environmental heterogeneity to provide a range of conditions. However, the investigator may prefer to examine a gradient of conditions (for example, a variety of habitat types within one segment or segments of different stream order) or compare a set of contrasting conditions (e.g., constrained vs. unconstrained, logged vs. unlogged, roaded vs. unroaded, grazed vs. ungrazed, burned vs. unburned, etc.) within the same stream or an otherwise comparable stream within the same ecoregion. A single, paired-comparison may be instructive for learning purposes but adequate replication is needed for research projects.

III. SPECIFIC METHODS

A. Attenuation of Solar Radiation/Shading

Select a series of riparian habitat conditions ranging from sunlit to heavily shaded or compare reference and treatment segments. Measure midday light levels, with a pyranometer or quantum sensor, in midstream and in the center of the riparian zone at multiple points along each stream-riparian segment and in an open (reference) area anywhere in the general vicinity. Also measure air and water or soil temperatures at depths of 0 and 5 cm at each of these locations. Determine the absolute and relative degree of shading and its effect on the thermal environment in each segment and compare the results between/among segments. Improvements to this basic approach would be to integrate values over a day, season(s), or year by obtaining representative sets of multiple measurements (e.g., hourly throughout a day) or through use of a data logger. In addition, the temperature measurements could be linked to microbial activity or (in terms of cumulative *degree days*) to leaf decay rates (see following).

B. Input and Decomposition of Coarse Particulate Organic Matter

The basic approach for quantifying CPOM input from the riparian environment to the stream is to: (A) Measure the mean amount of CPOM produced by each of the major plant species in relation to canopy volume (e.g., g dry mass (DM)/m^3 or g AFDM/m^3).

(B) Determine the density and canopy volume of each major plant species for each distinct riparian segment of interest (or for all of the different riparian habitat types represented in the zone of interest). An added refinement would be to stratify the taking of census data across the moisture gradient, extending from stream channel to outer edge of the riparian zone, to obtain mean values for each distance-stratum. (C) Calculate the total biomass of each major plant species in the area of interest. (D) Categorize the plant species according to litter-processing categories and determine the total biomass in each category.

Although the amount of litter reaching the stream channel generally is proportionate to the amount and species composition produced by the adjacent riparian plant community (Cummins *et al.* 1989, Swan and Palmer 2004), it often is desirable to separate the amount contributed to the stream from that remaining on the land. This can be done by measuring the amount of litter in the stream channel soon after leaf fall is complete or by measuring the amount remaining on the land after export by wind and gravity have ceased (e.g., in late fall, winter, or early spring after an extended period of wetting by precipitation has stabilized the litter) and calculating the unmeasured component by difference. In-stream measurements may be confounded by water-borne import or export of litter from outside the study area boundaries.

The specific methods described below focus on riparian shrubs of small to moderate size, as these are the predominant vegetation in many stream/riparian settings. However, though more challenging to implement, with appropriate adjustments the litter production of larger shrubs and deciduous and coniferous trees can be measured using similar procedures. For many purposes a stream segment 250-m long will be adequate for study, although appropriate adjustments should be made depending on the specific objectives of the study. For illustrative purposes, it will be assumed that the mean width of the riparian corridor is 20 m, evenly divided on each side of the 5-m wide stream segment.

Protocol for Measurement of Litter Produced by Each Major Plant Species

1. In the autumn, prior to leaf fall, encircle the bases of 5 or more shrubs of each target species with fine-mesh netting. The mesh size will depend on the smallest diameter of the leaves or needles to be collected but generally will range between 1 to 10 mm in diameter. Various materials may be used including orchard netting, seine netting, plastic window screening, or even bed sheets. Support the outside edge of the material with 46 cm (18″) surveyor's stakes driven 15 cm (6″) into the ground, fasten the netting to the tops of the stakes with thumb tacks or staples, and allow the intervening material to rest on the ground.

2. Cover each shrub with additional netting that extends down past the tops of the stakes and secure it to the stakes. In cases where this step cannot be accomplished, the exercise may proceed but with the realization that an unknown and variable portion of the litter harvest may be lost.

3. After leaf drop is complete, perhaps with the aid of vigorous shaking of the branches by the investigator, bundle up the leaves in the base netting; label it as to date, location, and height and diameter/width (depending on geometric shape) of the shrub; and return each bundle to the laboratory.

4. Air-dry the material and then dry it at 60°C until all moisture is removed — that is, no further weight loss occurs — and record the final weight. If facilities do not

permit drying of the entire bundle, subsamples may be taken of the air-dried material, weighed, dried, and reweighed to obtain a conversion factor for calculating the dry weight of the total.
5. Express the results for each shrub species of interest in terms of grams dry mass (or AFDM) per cubic meter of canopy volume, where plant volume is calculated from canopy height and width or diameter using the mathematical formula for the appropriate geometric shape (e.g., a sphere). Determine the mean value and standard deviation for each species.

Protocol for Determination of Plant Density and Total Canopy Volume and Calculation of the Total Litter Biomass

1. Use a suitable method for determining total plant density of each species for the entire study segment (e.g., $250\,\text{m long} \times (10 + 5 + 10\,\text{m wide}) = 6{,}250\,\text{m}^2$). For the purposes of the approach described here, a method such as the point-quarter method (Mueller-Dombois and Ellenberg 1974) is more suitable than one such as the line-transect method.
2. Calculate the total volume of each major plant species as the product of total density × mean volume.
3. Convert total canopy volume to litter biomass by multiplying by the mean amount of litter per cubic meter.
4. Although total loadings are instructive in their own right, for comparative purposes, results should also be expressed as mean densities (g/m^2) by dividing the total biomass values by the total area of study.

Protocol for Determination of the Total Biomass in Each Litter-Processing Category

1. Categorize the plant species according to decay rate (fast, medium, slow) and sum the results of the values derived above by category. Categorization can be done by means of empirically derived decay rates as described below in the decomposition section or by using published results referred to in Section II.
2. Determine the relative (%) contribution by each processing category and for the predominant species within each category.

Protocol for Separation of the Amount of Litter Contributed to the Stream from that Remaining on the Land

1. Collect all of the coarse litter from multiple (five or more) transects in the stream or on the land (e.g., 1-m wide bands across the streambed or riparian zone), place them in separate bags, and label.
2. Separate the leaves by species, combine into processing rate categories, determine a mean mass per m^2 for each, and multiply it times the area of the stream or riparian segment of interest. Subtract this value from the total value to obtain the value of the other portion (stream or riparian).

Protocol for Measurement of CPOM Decomposition Rates

1. Leaf decomposition methods are described in detail in Chapter 30. The procedure is to place packets of weighed leaves of a given species in the stream or on the riparian soil surface for a series of different exposure times.
2. Five-gram packs generally are used and the leaves are either fastened loosely together with monofilament or placed in mesh bags and tethered in the stream. If mesh bags or other enclosures are used, care must to be taken to use sufficiently large mesh openings to ensure aerobic conditions and allow access by shredding invertebrates.
3. Aquaculture cage netting having 5-mm openings has proven satisfactory. For teaching and some research purposes, leaves may be stockpiled in advance, air-dried, and stored in labeled plastic trash bags until needed. Generally, one or more bags of each species of interest will be needed. For the purposes of this protocol, it will be assumed that measurements will be made both in the stream and in its adjacent riparian zone.
4. Make up a set of leaf packs for each species of interest; each set generally will consist of 6–10 packs (3–10 gDM each) times the number of replicates desired (usually 3–5) for each habitat of interest (= a total of 36–100 packs for each species). Note: for short-term studies, such as a class laboratory exercise, fast leaves will prove the most satisfactory.
5. At the start of the study, disperse the entire set of packs throughout the study area and expose them to the stream or riparian conditions until they are retrieved. Allow 24–48 h for leaching of water-soluble substances before collecting the first subset of packs, then collect additional subsets every 150–300 degree-days, depending on the leaf-processing category of the leaf (the faster the expected processing rate, the shorter the thermal interval).
6. Degree-days are calculated by summing the mean diel (24-h) temperatures for each day of exposure; a miniature data logger, in a waterproof container, is ideal for obtaining precise values but a maximum-minimum recording thermometer will suffice. To determine the approximate sampling frequency: take the mean of the maximum and minimum stream/soil temperatures, measured over an interval of 24 h to several days at the time of the study, and divide it into the desired degree day value. For example, for a maximum temperature of 14°C, a minimum of 6°C, and a desired 150 degree-day exposure period the sampling interval would be 150°C days/10°C = 15 days. The intervals should be selected to give packs that have lost approximately 25%, 50%, and 75% of their initial dry weight after leaching.
7. At the time of removal, place a 250-um mesh dip net under the leaf pack, transfer the pack and net contents to a labeled plastic bag, and refrigerate until drying and weighing (60°C until a constant weight is attained). Rinse leaves over a sieve to remove sediment and invertebrates. Invertebrates can then be collected and saved in vials or bags for use in the next section. If samples cannot be processed upon arrival in the laboratory, samples should be frozen until processing can be done.
8. Calculate the decay rate (k) from the slope of the best-fit line in a semi-logarithmic plot of percent dry mass remaining versus exposure time (on x-axis) or as the least squares fit of the data to an exponential function: $W_f = W_i e^{-kt}$, where W_i and W_f

are the initial and final weights and t is the amount of time (days) leaves were in the stream.

$$-k = log_e \ (\%R/100)/t, \ \text{where } \%R \text{ is the percent remaining at any time } (t).$$

$$\%R = W(t_f)/W(t_i) \times 100 \tag{31.1}$$

The rate coefficient can be converted into mean daily %loss by:

$$\%R/\text{day} = (1 - e^{-k}) \times 100. \tag{31.2}$$

9. Leaf decomposition is strongly controlled by the thermal environment and temperature may explain much of the difference between riparian and stream decay rates (see preceding section on Attenuation of Solar Radiation/Shading). For comparative purposes, decay rates may be standardized for temperature by substituting degree days of exposure in place of time in the preceding plot or by using the relationship: $W_f = W_i e^{-kt}$, where $t =$ cumulative degree days.

General Assessment of Fungal and Detritivore Standing Crops

Leaves and other litter are primarily colonized by fungi, which soften the leaf tissue and make it attractive and suitable for ingestion by macroinvertebrate shredders (Kaushik and Hynes 1971). Fungal colonization and biomass in stream water or on riparian soil generally follow a succession of events over time as leaf decomposition progresses. This can be demonstrated through periodic examination of leaf surfaces after different exposure times or by measuring the fungus-specific, indicator-molecule ergosterol (Gessner and Chauvet 1994; see Chapter 15).

Stream macroinvertebrate shredders may be quantified using quadrat-sampling techniques and devices such as a Hess or Surber sampler or if in cobble-bed rivers the Hauer-Stanford net (Merritt and Cummins 1996; Chapter 25). Collect a minimum of five samples from each stream segment of interest, remove all of the shredders from each, determine the shredder biomass in each sample, and calculate the mean biomass and SD. In general, there should be a direct relationship between the amount of CPOM in a stream and the biomass of shredders. This can be tested with the data collected thus far.

It also is believed (Cummins *et al.* 1989) that shredders will maximize their biomass at the time of greatest availability of a litter in a given processing class that is in a state of decay that can support maximal growth (approximately the 50% weight-loss point). By initiating the measurement of CPOM decomposition rates (see previous section) at the time of maximum leaf drop and sampling shredder biomass at regular intervals until at least 50% of the weight of the leaf pack of the target species has disappeared, this hypothesis can be tested. Ideally, sampling intervals will be selected to yield leaf packs

and shredder biomass when approximately 25%, 50%, and 75% of the initial leaf pack biomass after leaching has been lost. However, if the timing is not known from previous study, it can be approximated from published values. For example, for fast-decaying plant species, sampling every 10–14 d over a two-month period would be adequate to encompass the entire decay sequence. In the case of medium or slow species, attaining the 50% loss point indicated above, possibly using a logarithmic scheme of increasing sampling intervals, should provide results satisfactory for illustrative purposes. Plot the mg of shredder biomass per gram of remaining leaves (y-axis) against the percentage of leaf mass remaining (x-axis). If the hypothesis is supported, the peak shredder biomass will occur at approximately the 50% weight loss point.

Similar procedures and rationale can be used in investigating the riparian soil fauna. One approach is to collect soil plugs using a bulb planter or special soil corer and physically remove all of the shredder-type organisms (e.g., earthworms) from each core, measure their biomass, and relate it to riparian litter standing crops or decay rates as just described.

C. Transfer of Dissolved Organic Matter and Nutrients from the Riparius to the Stream

As described in Section IIC, water is the principal mechanism for transporting DOC and nutrients from the riparius to the stream and hence their transfer is closely linked to the hydrologic cycle (Figure 31.1). In addition, riparian zones can be potential sources or sinks for DOC and nutrients depending on redox conditions (Mulholland 1992). The approach taken here is to isolate each of the important compartments along the hydrologic cycle in which DOC and nutrients occur and measure their concentrations. After collection, all water samples can be analyzed according to APHA standard methods (1998) for nitrite, nitrate, total Kjeldahl-N, ammonium-N, total-P, orthophosphate-P, and DOC, depending on the research objectives. The study of nitrogen transfers in riparian environments is especially valuable because of the critical role that nitrogen plays as a limiting nutrient and because its various forms are diagnostic of particular states (aerobic versus anaerobic) and biological transformations occurring there.

Precipitation can be measured with a rain gauge and samples collected in clean polyethylene bottles that are placed level on the ground and covered with mesh to keep out insects and large debris. Samples should be collected following a rain event.

Surface runoff measurements are made with surface water collectors consisting of polyethylene bottles placed into holes in the soil in an inverted position with a rectangular slot cut into the uphill side at ground level. A plastic sheet is attached to the bottom of the slot and spread uphill to direct flow into the bottle (Peterjohn and Correll 1984). For maximum information, each runoff event should be sampled separately.

Groundwater measurements can be made through collection of water samples from PVC wells placed in the riparian zone (see Chapter 6). PVC slotted wells should be 5–7.5 cm (2-3″) in diameter, contain well screens, and have PVC plugs. Location of wells depends on the question being addressed and hydrogeology of the area. In wetland areas it is easier to place wells at varying depths than it is in areas outside wetlands. In wetland areas, wells can be placed along transects adjacent to the stream. These transects should be around 50 cm apart and contain wells in rows, about 5–10 wells in each transect. Wells can also be randomly placed in the riparian area within 1–5 m of the stream. Installation of wells along transects from inland to the stream edge allows examination of water as it moves across the soil/stream interface. This is most easily done with streams

that have a large floodplain. A topographic map of study streams could help establish transects. For different methods of well installation see Chapter 6. Wells should be bailed before sampling and allowed to recharge. Temperature, conductivity, pH, and dissolved oxygen should be measured before water samples are collected. This can be done using a YSI instrument or any other portable field probe. Samples can be collected from wells using a bailer, filtered and stabilized in the field, and returned to the laboratory for analysis employing standard methods (APHA 1998). After filtration, samples can be analyzed for nitrite, nitrate, ammonium-N, total-P, orthophosphate-P, and organic matter concentrations (DOC, DON). Analyses of the various forms of nitrogen can be used to detect patterns in soil biogeochemistry from different environments to measure the effects of N inputs.

Soil measurements can be made from riparian and upland areas with a soil corer, dried at 60°C, and analyzed for nitrate and ammonium (Pace *et al.* 1982). These results can be compared to stream and well water concentrations to determine if correlations are present and to determine whether the soil is a potential source or sink. The latter will vary with geology (e.g., valley confinement and rock type) and plant species composition of the watershed and riparian areas (e.g., xeric versus mesic and grasses, shrubs, trees and N-fixing plants). For a more detailed analysis of soil, microbial biomass can be estimated though several methods, which include staining and counting of microbial cells; physiological parameters, such as ATP, respiration and heat output; or fumigation techniques (Pace *et al.* 1982). Further understanding of stream/riparian zone biogeochemistry and microbial activity can be gained though measurement of redox conditions (Hedin *et al.* 1998).

A soil's capacity to transform organic nitrogen in soil organic matter to inorganic nitrogen (*nitrogen mineralization potential*) is often used as an index of the nitrogen availability (Robertson *et al.* 1999). Nitrogen mineralization releases large amounts of ammonium and measures the net increase in both ammonium and nitrate in soil, since any nitrate formed must first have been NH_4. The relative availability of N can be measured with ion exchange resin bags (Binkley and Matson 1983, Binkley *et al.* 1986). The resin bags mimic nutrient uptake by plant roots by the adsorption and accumulation of nitrate and ammonium to the resin beads forming an ionic bond with + or − charged particles on the beads. Resin bags can be placed in transects that run perpendicular to the stream or in random locations in the riparian zone, and in upland areas, depending on the question being addressed. Resin bags placed in the riparian zone can be compared in terms of N pools with those placed in the uplands; this will provide insights as to the processes taking place in the riparius (e.g., Frank *et al.* 1994).

Resin bags are prepared by placing mixed-bed ion-exchange resin in nylon stockings (see Binkley *et al.* 1986). After the bags are prepared, store them in a Ziploc® and label with the name/number of the batch (each bottle of resin constitutes a batch). Record the number of the resin bag before placement in soil.

For placement in soil, cut out an area about 10 cm deep then at an 90° degree angle, cut into the wall of the hole (about 5 cm from the bottom), making a shelf for the resin bag to sit on without disturbing the soil above (this is very important). After resin bags are in place, fill in the hole and mark with surveyor's flagging. Time of incubation will vary depending on environment of placement (wet versus dry). Incubation periods can vary from weeks to months. When removing resin bags, take care not to damage the resin bag. Resin bags should be placed in Ziploc® for transport to laboratory, air-dried, and the resins extracted with 1N KCL (1g resin/15 mL 1N KCL). Filtration of extract must be done 24 hours after resin is mixed with KCL and shaken. Another resin

bag can be placed on the same shelf in the original hole if the soil is not disturbed, to look at long-term or seasonal processes. The extracts are analyzed for ammonium and nitrate by standard soil chemistry methods (Pace *et al.* 1982). Results are reported as resin ammonium and nitrate accumulation in mg/per day. Competition with plants and soil microbes may strongly reduce available N. Increases in water flow to the resin bags may increase ammonium capture more than that of nitrate (Binkley 1984).

Stable isotope analysis (SIA) can be used to examine trophic status and energy flow pathways (Chapter 27). Representative qualitative samples or material generally will suffice for these analyses. For this Chapter SIA will be used to determine linkages between riparian vegetation, soil, groundwater, stream water, invertebrates, and the effects of MDN to better understand riparian-stream processes. Using a combination of C and N isotopes for all samples collected will help determine organic matter transfers and give insight into trophic structures. There are numerous laboratories throughout the United States and Europe that can run samples for stable isotope analysis at reasonable prices (about \$12–\$30/sample). Results obtained are expressed as $\delta^{13}C$ and $\delta^{15}N$ using the following equation:

$$[(R_{\text{sample}} - R_{\text{standard}})/(R_{\text{standard}}) \times 1000] \tag{31.3}$$

Where R is the ratio of ^{15}N to ^{14}N and the standards are Pee Dee Belemnite (PDB) carbonate for $\delta^{13}C$ and atmospheric N for $\delta^{15}N$.

Algae, leaf, and soil samples should be dried, ground, and placed in labeled glass vials until analysis. Qualitative samples of algae can be collected by methods described in Chapter 16; the material should be placed into vials and dried at 60°C. The substrates where algae are collected should be similar for each replicate. Leaves can be collected from vegetation during the senescent period or collected from the ground or the stream. Extra leaf packs can be placed in the riparian zone and in the stream for separate SIA analysis. Soil samples can be collected from upland areas and compared to riparian soil samples. Invertebrates should be allowed to clear their guts before they are dried and ground. Gut clearance can be done by separating the invertebrates, to avoid predation, and then leaving them overnight in aerated flasks. Clear guts will eliminate measurement of unassimilated food particles in the gut. Water samples should be placed on ice immediately and kept cold until analysis or frozen if analysis cannot be completed within a few days. Water samples also can be analyzed for $\delta^{15}N\text{-}NO_3$ and $\delta^{15}N\text{-}NH_4$.

Carbon and nitrogen isotopes are useful indicators of trophic status (Figure 31.2). Comparison of $\delta^{15}N$ values among vegetation, soil, and water samples can provide information on the processes taking place. Separation in $\delta^{13}C$ for consumers eating different sources (periphyton versus detritus) should be easily detected. Finlay (2001) found that, in forested headwater streams, $\delta^{13}C$ in algae was distinct from terrestrial detritus $\delta^{13}C$. There also can be 3–5% enrichment in $\delta^{15}N$ of consumers relative to their food (Peterson and Fry 1987). Analysis also can be done where a marine signature is expected, to investigate the effects that salmon may have on riparian-stream processes. Collect samples from all compartments, as previously described, and compare the results with those collected from areas where no marine signature is present. Communities that are alder-dominated versus communities where alder is not present should also show different signatures in the soil and leaf chemistry addressing the effects that N-fixers may have on processes.
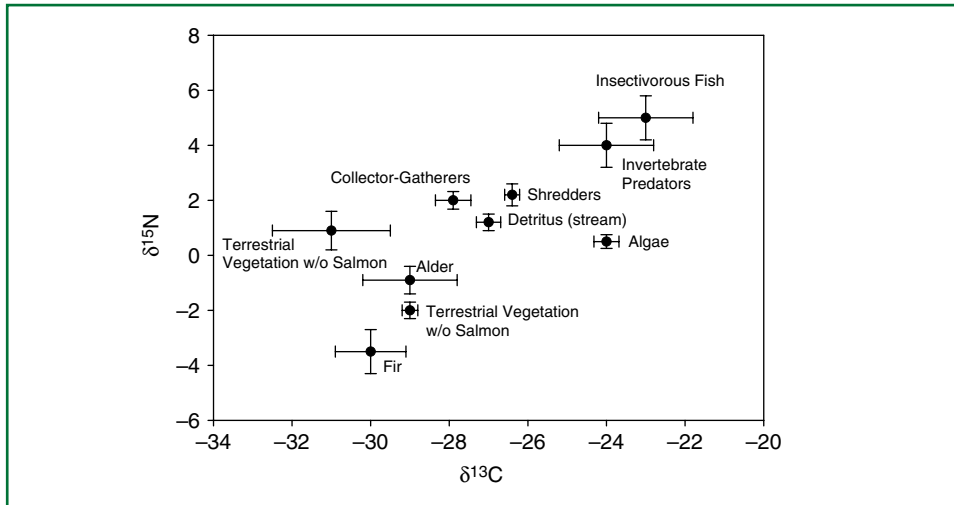
FIGURE 31.2 Sample stable isotope diagram of $\delta^{15}N$ and $\delta^{13}C$ for components of stream and riparian areas. Nitrogen isotopes are indicators of trophic level (2% fractionation for each level), while carbon isotopes indicate which plants (terrestrial and aquatic) are potential sources for consumers.

IV. QUESTIONS

1. How do solar input and canopy cover change as a stream increases in size (1–4 order), or how do they differ within a stream (e.g., adjacent to meadow, forest, shrubland, agricultural field, etc.)? Do these values correlate with CPOM input?

2. Is there a relationship between solar input and stream temperature, dissolved oxygen, or periphyton? Does stream temperature change with varying amounts of total solar input? How do these relationships change quantitatively on an hourly, daily, or seasonal basis and between reference and treatment sites of your choosing? Is there a relationship between % canopy cover and periphyton chlorophyll *a* in these streams?

3. What differences are found in riparian litter input and decomposition rates among sites (such as those listed in question 1), and how does this relate to soil and groundwater properties?

4. Calculate litter inputs from the riparian zone into the stream, total litter biomass, and then separate the litter into processing categories. How do these measurements compare with those of CPOM collected in the stream? What relationships and patterns are present and how will these patterns vary with stream order, different species of riparian vegetation, or land-use?

5. What differences in water quality (DO, temperature, pH, conductivity and nutrient concentrations) did you find between the stream and groundwater and what may cause these differences? What differences were there between nutrient concentration found in groundwater and stream water and how does this vary with different topography and land-use?

6. Did you find differences in nitrate and ammonium among wells located in the lateral transect across the riparian zone? How do you account for these differences?

7. What differences are found seasonally in the riparian soil N, as reflected in resin bags, and how does the N content change at increasing distances away from the stream to upland areas?

8. What differences are found in soil and groundwater properties in riparian areas dominated by alder, those that are mixed with alder, and those without alder present?

9. Does gut content analysis of stream or riparian consumers that feed on leaf litter vary with riparian deforestation and or increases in sunlight?

10. In what ways do changes in land-use (deforestation, agriculture, and urban) affect stream/riparian interactions? How did you come to these conclusions (what measurements did you use) and what management recommendations could you make?

11. What changes occur in $\delta^{15}N$ values for leaves collected from vegetation during senescence, those decomposing on the forest floor (leaf packs) and those in the stream (leaf packs)? How do these values relate to soil properties and in-stream water chemistry?

12. Using stable isotope analysis, what differences did you find between riparian vegetation, periphyton, and macroinvertebrates in streams with marine-derived nutrients and those without? How do you explain these differences?

13. What do you conclude from SIA analysis on $\delta^{15}N$ and $\delta^{13}C$ regarding the dominant energy source to invertebrate consumers in the stream and riparian habitats you examined? How could the timing of sampling during the year influence the results you might find?

V. MATERIALS AND SUPPLIES

The investigator is encouraged to exercise a bit of ingenuity in acquiring the materials and equipment used in the procedures described in this chapter. Considerable saving can be made and a broader array of solutions obtained by using sources other than specialized scientific-equipment suppliers. Local sources such as building supply, farm, general merchandise, hardware, and lawn & garden stores can supply many of the needed items either directly or for subsequent fabrication by the investigator. Additional sources are to buy in bulk from the manufacturer or primary supplier or to purchase used or surplus materials from a second-hand or salvage store (e.g., plastic insect netting from an army/navy surplus store). However, for those with limited time or abilities or with unlimited bankrolls, scientific products suppliers will be the obvious source of choice.

Field Materials and Equipment

Attenuation of Solar Radiation/Shading

> Pyranometer or quantum sensor
> Thermometer
> Spherical densiometer
> Solar pathfinder

Input and Decomposition of Coarse Organic Matter

> Netting, fine mesh (in bulk quantity)
> Surveyor's stakes (46 cm)

Thumbtacks or staples
Labeling materials
Meter stick and tape
Leaves
Thermometer, recording type
Collecting nets (250 μm mesh recommended)
   Dip net
   Surber or Hess net
Plastic bags
   Large (garbage bag) size for bulk leaves
   Small (sandwich bag) size for soil samples, etc.
   Vials (e.g., scintillation type)

Transfer of Dissolved Organic Matter and Nutrients

Polyethylene bottles
Whirlpaks
Meter tape
Rain gauge
Fine mesh netting (small enough to keep out debris from polyethylene
   precipitation bottles)
Scintillation vials
Plastic sheeting
Surveyors flagging
Sharpie
Whatman filters or Millipore HA membrane filters (0.45 μm)
Ziploc® bags
PVC wells 5–7cm (2–3″) diameter
Well screens
PVC plugs
Well bailer
Auger or fence post driver (for placement of wells)
YSI or other portable field probe
Soil corer
Periphyton sampling equipment (Chapter 16)
Surber or Hess Sampler (Chapter 20)
Mortar and pestle or Willey Mill

Laboratory Materials and Equipment

Input and Decomposition of Coarse Organic Matter

Monofilament line or plastic mesh for leaf pack construction
Laboratory balance
Drying oven (60°C, large capacity)

Transfer of Dissolved Organic Matter and Nutrients

Drying oven
1M KCL
Resin beads (Supelco Corp., MTO-Dowex®)
Nylon stockings
Flask (large enough to hold resin extract)

Parafilm
Vacuum filter
Whatman filters #1, 70 mm diameter

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Effects of Nutrient Enrichment on Periphyton

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I. INTRODUCTION

Definitions of *periphyton* vary within the literature. Here we refer to it as microfloral growth upon substrata (Wetzel 1983). Stream periphyton communities are affected by a complex array of interacting factors including nutrient and toxicant loading, light, temperature, water velocity, and grazing pressure. Experimental hypothesis testing is essential for understanding periphyton development and production in natural systems. Control of environmental variables and experimental isolation of regulating mechanisms can be a difficult task in the field, particularly in streams. The more control exerted by the investigator, the more replicable the result, but the less applicable to natural systems. On the other hand, less-controlled field experiments and observations may accurately describe the current periphyton community at a particular site, but yield little insight into what factors control community development. Furthermore, *in situ* manipulations are difficult to replicate under temporally and spatially varying background conditions.

Factors controlling periphyton growth in streams are poorly known compared to our knowledge of production limits in lakes. The unambiguous demonstration of nutrients limitation of algal periphyton production in a stream ecosystem is difficult for several reasons. A very low concentration of dissolved nutrients in overlying water may meet periphyton requirements due to the large volume of constantly renewed water. Also, nutrient levels

may be high at certain times of the day, during storms, or seasonally when allochthonous materials (e.g., autumnal leaf fall) release nutrients in high pulses.

Nutrient regulation of periphyton growth and production has been addressed using several different approaches listed here in order of increasing scale: (1) point source manipulation of nutrients via nutrient-diffusing substrata, (2) enrichment of the water using flowthrough enclosures, (3) whole stream manipulations, and (4) integrated bioassays that combine bioassay techniques, allowing comparison of algal growth response across different scales. Each approach has advantages and disadvantages, so the most appropriate method will depend on the scale at which the investigator is addressing nutrient limitation (Pringle *et al.* 1988), the nature of the stream system under study, and the tractability of using a specific approach in that system. Below we provide important background information on each of the above approaches.

A. Point Source *In Situ* Nutrient Manipulations

Point source nutrient manipulations allow testing of periphyton response *in situ* without the artificiality of enclosures. Such manipulations sacrifice the control of an enclosure for the more natural interaction with the total aquatic environment. Point-source manipulations such as nutrient-diffusing substrata can be advantageous in that they introduce minimal solutes to the environment, an important consideration when the same habitat is simultaneously utilized by numerous investigators. Such manipulations simulate natural nutrient-rich substrata that act as point sources of nutrients for attached algae. For instance, many larval Chironomidae consolidate sand grains into tubular-shaped retreats. The excreta of retreat-dwelling larvae provide a direct nutrient source to tube colonizing periphyton (Pringle 1985). Vascular macrophytes, wood debris, and other particulate inputs also constitute natural nutrient-diffusing substrata. Several types of nutrient-diffusing substrata, generally using an agar matrix, have been developed to test periphyton response to *in situ* point sources of specific elements or compounds.

Pringle and Bowers (1984) consolidated, washed, and sterilized sand from the streambed into petri dishes with agar solutions enriched with phosphate and nitrate to simulate the structure and texture of nutrient-rich chironomid tubes that are composed of sand grains. The growth response of algae colonizing these circular "bricks" was assessed. A different approach uses terracotta clay flower pots filled with nutrient-rich agar and sealed with plastic petri dishes (Fairchild and Lowe 1984, Fairchild *et al.* 1985). Clay pots provide a hard surface similar to an epilithic (rock) habitat, as opposed to sand-agar substrata, which are more representative of an epipelic (silty) habitat. Tate (1990) modified the clay pot technique for use in stream systems to minimize variable current regimes: clay flowerpot saucers were filled with agar, sealed with Plexiglas, and installed *in situ* with their bottom surface parallel with the current flow to provide a horizontal surface for algal periphyton colonization.

The aforementioned nutrient-diffusing substratum techniques have proven to be effective tools in (1) evaluating local nutrient recycling processes (Pringle and Bowers 1984, Pringle 1990), (2) validating theoretical models (Fairchild *et al.* 1985), (3) detecting nutrient-limiting factors in a *shot-gun* approach (Lowe *et al.* 1986, Pringle *et al.* 1988), and (4) supplementing additional bioassay techniques (Grimm and Fisher 1986, Pringle 1987, Tate 1990).

A major limitation of the aforementioned nutrient-diffusing substratum techniques is the inconsistancy in the rate of nutrient release (Pringle 1987, Brown *et al.* 2001).

Nutrients are released in a high initial pulse with release rates decreasing exponentially through time. If algae responds to this initial pulse and then is sloughed off in the course of the experiment, nutrient effects may not be measured by the investigator on later sampling dates. In addition, (1) terracotta clay pots and saucers contain large quantities of iron, calcium, and aluminium (which bind phosphorus) and they can irreversibly sorb large quanties of phosphorus (e.g., Brown *et al.* 2001); and (2) variability in saucer composition and diffusion properties makes treatments difficult to replicate.

A more recently developed nutrient-diffusing substratum technique, developed by Matlock *et al.* (1998), provides for a more consistent rate of nutrient release through time. This passive nutrient-diffusing substratum technique, coined the *Matlock Periphytometer*, measures periphyton response to passive diffusion of nutrients through a biofilter and glass fiber filter. Nutrients are contained in 1 L polythylene bottle reservoirs capped with a biofilter membrane and glass fiber filter. The biofilter membrane allows for nutrient diffusion while the glass fiber filter provides a substrate for periphyton colonization. Harvesting of the entire glass fiber filter allows for complete recovery of periphyton for chlorophyll *a* analyses. [Periphyton colonizing terracotta pots often imbed themselves in the substrate and estimates of periphyton recovery efficiencies (i.e., from sampling semiporous media through scraping) range from 50 to 80% (Cattaneo and Roberge 1991)].

Two different point-source nutrient bioassay approaches are presented following in the *methods* section: (1) a basic method designed for student project-level investigations using nutrient-diffusing terracotta clay pots (complete methods provided) and (2) a more advanced method that employs the *Matlock Periphytomter* (detailed methods provided elsewhere).

B. Flow-Through Enclosures

Flow-through enclosures (i.e., flumes) can provide much flexibility for experimental manipulation, particularly for long-term studies. They serve as a valuable tool for isolating and modifying aspects of the physical, chemical, and biological environment of running water systems and for providing within-system replication. Such partially open enclosures are essential for long-term manipulation to minimize enclosure effects that could result in development of a unique and unrepresentative community. However, even partial enclosure of a natural system with walls may create a different environment than the unenclosed, natural system.

Partially enclosed flumes with a sealed or open bottom may be placed within the stream channel or may be located stream-side and receive stream water pumped from the channel. Nutrients are added to the water at the head of the flume. Flume channels located within the stream channel (Triska *et al.* 1983) or streamside (Rosemond 1993, Rosemond *et al.* 1993) have been used successfully to examine algal growth response to nutrient additions on either natural or artificial substrata. A classic study that used flumes *in situ* in a temperate, coastal-rainforest stream was conducted by Stockner and Shortreed (1978), who found that 3x increase of both NO_3 and PO_4 over ambient concentrations resulted in higher algal growth than when PO_4 alone was added.

An *in situ* flow-through flume system, based on the method of Peterson *et al.* (1983), is presented in the *methods* section as an example of a technique that is suitable for both student project-level investigations and more advanced studies.

C. Whole-Stream Manipulations

The least controlled of all approaches to determine nutrient effects on periphyton is whole-stream nutrient enrichment. This approach uses *in situ* hydrologic conditions for nutrient dispersal, and the mosaic of channel conditions as the experimental matrix. Flow-through enclosures, discussed earlier, eliminate or control many hydrologic factors, while whole stream manipulations realistically integrate in situ physical, chemical and biological factors that determine periphyton response. This realism comes at some cost since the measured nutrient response is that of the total system, not just periphyton. Total system response may be determined from upstream-downstream differences in mass of injected nutrient (Triska *et al.* 1989a, b) or as nutrient uptake length (the average distance a nutrient molecule travels prior to uptake). The latter metric is determined from concentration decline of added nutrients corrected for groundwater inflows, over several stations along an experimental stream reach (Solute Transport Workshop 1990). For long-term nutrient injections (>1 week), a more quantitative determination of periphyton response to nutrient addition may be obtained by incorporating other approaches outlined earlier. For example Triska *et al.* (1989) incubated artificial substrates (sandblasted acrylic plastic slides) above and below the site of nutrient enrichment several weeks prior to the enrichment. During, and at the conclusion of, the enrichment periphyton that had colonized slides from both control and treatment reaches were compared using biomass and chlorophyll analysis. In the same study, closed recirculating chambers containing natural substrata were also used to compare periphyton primary production and respiration above and below the site of nutrient amendment. Artificial and natural substrata can also be used to determine shifts in community structure resulting from nutrient amendment. For logistical reasons (e.g., the mass of injectate required), whole system manipulations are typically conducted in low order streams at base flow.

Whole stream manipulations are a useful, versatile tool in studies of nutrient dynamics in channel and hyporheic zones, at both background and nutrient amended levels. Most recent applications involve determining uptake length at background or quasi-background conditions. As noted earlier, nutrient uptake is by both autotrophic and heterotrophic components of the stream ecosystem. Their respective roles will vary both by site and shifting environmental factors such as canopy cover, season, discharge, velocity, nutrient concentration, etc. The most quantitative whole system application is injecting nutrient isotopes in conjunction with a conservative tracer (e.g., chloride, bromide, or the dye rhodamine WT). Where possible, isotopes are the preferred nutrient source, since amended tracer does not significantly increase background concentration. As a result isotopic tracers are less likely to saturate biotic uptake capacity. Isotopes are very expensive however, limiting their application to very small streams. When the isotope is radioactive (e.g., ^{32}P) permission to introduce radioactivity to the natural environment is a major impediment. For N-cycling studies, ^{15}N is non-radioactive, and numerous studies have taken place throughout the United States, using ^{15}N-ammonia or ^{15}N-nitrate. The great expense of the isotope and subsequent sample analysis have prevented the technique from becoming routine. A more economic substitute is nutrient amendment that results in only small increases of the target nutrient above background concentration. A comparison of ^{15}N ammonia studies to non-isotope injections found longer uptake length with non-isotope nutrient addition, as expected (Mulholland *et al.* 2002). However results from both approaches were comparable if nutrient additions remain as low as possible. Additional analysis of the same experimental data plus that from whole stream experiments in a prairie stream, indicated that N-uptake was a function of concentration

(Dodds *et al.* 2002). They further found that extrapolation of uptake measured in a series of whole stream amendments could be used to determine ambient channel uptake. Even a series of short-term whole system manipulations requires significant analytical capability for the chemical tracers and nutrient samples. In low solute streams however, conductivity increases of the halide tracer has been used to calculate the hydrologic parameters and minimize analytical costs.

In the final exercise described in the Specific Methods section, we suggest a simplified approach that requires minimal nutrient sampling and that is targeted toward periphyton response.

D. Integrated Bioassays

Integrated bioassays, which combine two or more different techniques, allow comparison of nutrient effects on algal periphyton across different scales. For example, Pringle (1987, 1990) combined a nutrient-diffusing substratum technique (sand/agar in petri dishes; Pringle and Bowers 1984) with flow-through bioassays to (1) experimentally differentiate between algal growth response to nutrients derived from the substratum versus those introduced from the water column, (2) examine the responses of specific algal taxa to enrichment of the water versus the substratum, and (3) compare the effectiveness of flow-through systems and nutrient-diffusing substrata as *in situ* bioassay methods. Periphyton growth responded to combined influences of water and substratum enrichment in an additive or synergistic manner, depending on the types of nutrients (N, P) added from each source. When NO_3 was added to the substratum and PO_4 to the water, algal growth response was synergistic (Pringle 1987). Furthermore, specific algal taxa exhibited different responses to enrichment of substratum versus water.

This chapter describes *in situ* field methods for quantitatively assessing algal growth response to nutrient additions over different spatial and temporal scales: (1) point-source nutrient enrichment using two different nutrient-diffusing substratum techniques, (2) nutrient additions to the water using a flow-through mesocosm technique, and (3) whole-stream nutrient enrichment. These methods can be combined in various permutations to examine nutrient effects on algal periphyton across different scales.

Specific objectives of this chapter are to (1) introduce the concept of nutrient limitation of algal periphyton growth; (2) demonstrate how to assess effects of nutrient enrichment on algal growth, as measured by the accrual of algal standing crop (i.e., chlorophyll *a*, ash-free dry mass); and (3) to illustrate advantages and disadvantages of different techniques applied at different spatial scales.

II. GENERAL DESIGN

In this chapter we evaluate the response of algal standing crop accrual, as measured by chlorophyll *a* and ash-free dry mass (AFDM), to nutrient manipulations using one (or more) of three specific methods. Each of the methods involve *in situ* field experiments designed to span a five-week time frame. All field experiments require regular visits to the stream study site (i.e., every two to three days for Exercise 1; every day for Exercises 2 and 3) for collection of water samples for background nutrients, removal of debris from artificial substrata, refilling nutrient reservoirs, and calibrating nutrient release rates. Analyses of nutrients (i.e., NO_3 and PO_4) are explained elsewhere in this book (Chapters 8 and 9).

In choosing a method, researchers are encouraged to analyze the advantages and disadvantages of each approach in terms of (1) the scale at which they wish to evaluate nutrient limitation of algal growth and (2) the tractability of the methods in light of the field sites available. The first two methods are most easily applied to third- to fourth-order streams, while the third exercise is best applied to first- and second-order streams.

A. Site Selection

Wadeable second- to fourth-order streams with relatively low ambient levels of nitrate ($NO_2 + NO_3 - N$) and phosphate (SRP) are ideal for the methods in this chapter. Within the study stream, a sunny reach (preferably <40% canopy cover), with a relatively simple channel (straight, uniform depth, and substratum type, sparse woody debris), should be selected. The length and width of the experimental reach should be chosen to accommodate the bioassay apparatus/materials associated with a given exercise. For instance, if Basic Method 1 is used, the ideal site will provide suitable space for the installation of 48 clay saucers in 12 groups of four treatments.

B. General Procedures

Procedures for determining algal response to nutrient treatments will be assessed through analysis of chlorophyll *a* and AFDM. Chlorophyll *a* samples should be vacuum filtered (Whatman GF/F filters), ground in 9 mL buffered acetone, and analyzed spectrophotometrically (APHA *et al.* 1992). Ash-free dry mass samples should also be filtered onto preweighed Whatman GF/F filters (or equivalent), dried for 24 h, weighed, ashed, rehydrated, dried for 24 h, and reweighed (see Chapter 17 for detailed protocols).

Background stream nutrient chemistry must be collected every two to three days in acid-washed (10% HCl) and thoroughly rinsed 125-mL polyethylene bottles for analyses of NO_3 and soluble reactive phosphorus (SRP).

III. SPECIFIC METHODS

A. Basic Method 1: Nutrient Limitation of Algal Growth Using Nutrient-Diffusing Substrates

This method uses a nutrient-diffusing substratum technique designed by Tate (1990; Figure 32.1). This terracotta flowerpot-saucer technique has been chosen because of its minimal expense and the ease and facility with which it can be constructed and installed. It is our opinion that this technique can be useful in a *shotgun* approach to assessing algal nutrient limitation by examining relative differences between treatments, but its limitations must be carefully considered when interpreting data and making conclusions (Brown *et al.* 2001). Other approaches are recommended for quantitative assessment of algal response to a relatively constant point-source release of nutrients (e.g., Matlock *et al.* 1998; see Advanced Method 1).

In the basic methods presented here, nutrient-diffusing substrata are incubated within the stream in standardized conditions of current velocity, depth, and canopy cover. They are then retrieved after one-, two-, and three-week time periods and processed for assessment of chlorophyll *a* and AFDM.
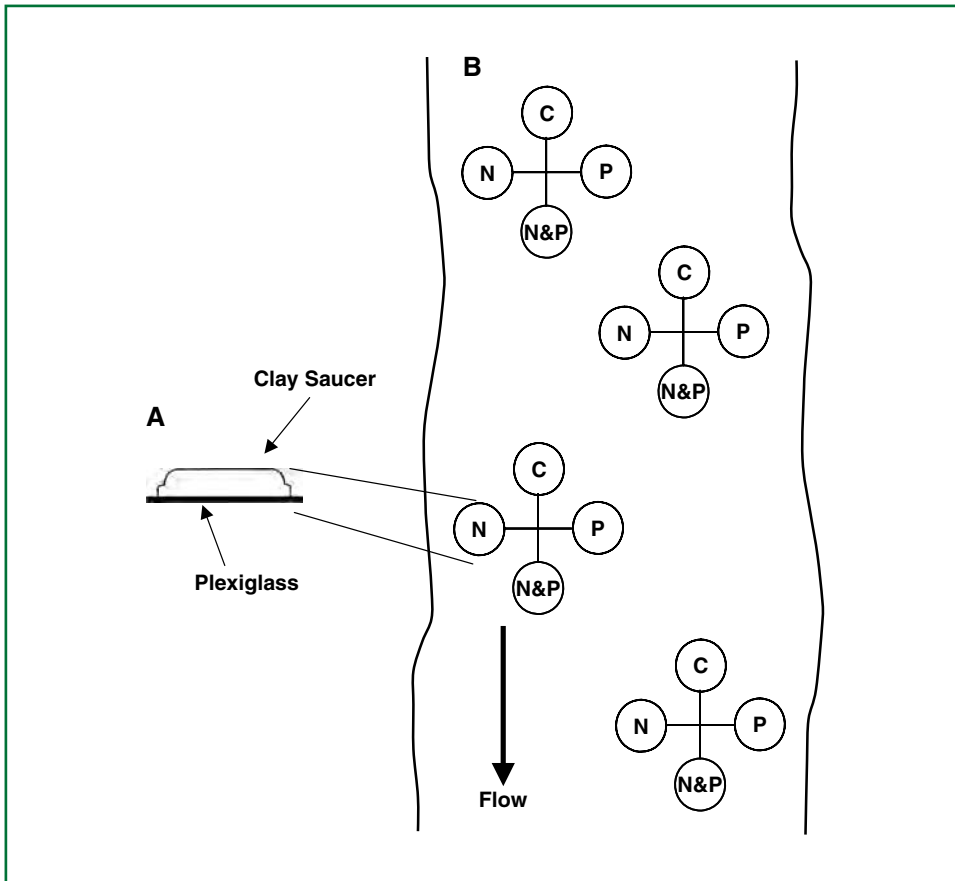
FIGURE 32.1 A. Nutrient-diffusing clay saucer. B. Orientation of blocks of nutrient-diffusing clay saucer treatments with respect to the current direction in stream. C, control; N, nitrate; P, phosphate; and N+P, nitrate and phosphate.

1. Construct nutrient-diffusing artificial substrata out of 10.0–11.0-cm-diameter (at base) clay flowerpot saucers glued to Plexiglas plates. Fill four sets of 12 clay saucers with 225 mL of 2% agar solutions. Four batches of agar should be prepared for the following treatments, with each treatment represented by 9 saucers: (1) N enrichment, 0.5 mol/liter $NaNO_3$; (2) P enrichment, 0.1 mol/liter KH_2PO_4; (3) N+P enrichment, 0.5 mol/liter $NaNO_3$ and 0.1 mol/liter $KR^{\wedge}PO^{\wedge}$ (4) C, control with no nutrients (i.e., unenriched agar only). Tops of saucers are sealed with waterproof adhesive to Plexiglas squares (15×15 cm) with a hole in one corner so that the plexiglas square can be attached to a wooden frame or staked to the stream bottom.
2. While many previous studies using this and similar techniques have measured significant nutrient release from terracotta substrata, release rates have been found to be inconsistant through time (high initial pulse followed by exponential decline) and varies, depending on the type of terracotta that substrates are constructed of. It is thus important to measure nutrient release rates.

3. To assess nutrient release rates from terracotta saucers, construct an additional three replicate saucers for each treatment and incubate them in separate beakers of distilled-deionized water. Maintain beakers at ambient temperatures of study stream water and agitate/mix the water several times each day. Water from each of the beakers should be replaced daily and concentrations of NO_3 and PO_4 measured at one- to five-day intervals for 21 days.

4. An alternative approach, which provides for longer-term nutrient release of nutrients from terracotta saucers, is the use of slow-release fertilizer pellets. For example, using this technique in a simple two treatment experiment (i.e., control and a combined N+P treatment), clay pot saucers can be filled with 200 g of Osmocote[R] slow-release fertilizer pellets (17:6:12) for the N+P nutrient treatment, and 200 g of granite gravel for the control treatment, and topped with 3% agar. The gravel fill is used to provide equivalent weight to all saucers to keep them flat against the stream bed (Dye 2005).

5. Data sheets should be prepared for data collection associated with field installation and should include columns for recording saucers number and treatment, depth and current velocity for each saucer location, extent of canopy cover at field site, retrieval dates, and chlorophyll *a* and AFDM values, which will be expressed in units of mg/m^2 surface area of artificial substratum surface (see Chapters 12, 13, and 17 for details of determining AFDM values).

6. Saucers should be attached to wooden frames constructed in a diamond shape configuration, with a C saucer placed upstream, N and P saucers placed side by side downstream from C, and an N+P saucer at the downstream end, to minimize contamination among treatments (Figure 32.1, after Tate 1990). The wooden frames with saucers should be mounted in the stream bottom with stakes and positioned in a randomized block design within standardized conditions of current velocity, depth, and canopy cover. The latter may be measured with a spherical densiometer.

7. Collect four sets of saucers after one, two, and three weeks. Place each saucer into a separate Ziploc® and carefully transport to the laboratory. Scrape algae from the exposed top flat surface of each saucer (using toothbrushes and/or razor blades), dilute in a known volume of distilled water (e.g., 100–400 mL) in a 500-mL beaker, mix with a magnetic stirrer, and subsample (e.g., 20–50 mL) for chlorophyll *a* and AFDM. Chlorophyll *a* and AFDM samples should be processed according to methods just described and in detail in Chapter 17. The exact amount of the chlorophyll *a* or AFDM subsampled should be determined by the researcher in the laboratory based on the density of algae within the algal homogenate.

Alternate Technique—Passive Nutrient-Diffusing Substratum

The passive nutrient-diffusing substratum technique developed by Matlock *et al.* (1998) measures periphyton response to passive diffusion of nutrients through a biofilter and glass fiber filter attached over the mouths of 1 L polyethylene bottle nutrient reservoirs. To assess potential nutrient limitation of periphyton, bottles are mounted on a frame and incubated *in situ*. Glass fiber filters and their attached flora are retrieved through time and analyzed for chlorophyll *a*.

The technique has advantages over the nutrient-diffusing terracotta saucer technique because of its more consistent release of nutrients and complete recovery of algal periphyton growing on glass fiber filters. Complete details for construction of this bioassay

apparatus can be found in Matlock *et al.* (1998). Modifications of this technique, using 20 mL scintillation vials instead of 1 L bottles, have been used successfully to assess nutrient limitation of algal periphyton in streams draining Georgia's coastal plain (Carey 2004).

B. Advanced Method 1: Nutrient Limitation of Algal Growth Using Flow-Through Enclosures

In this exercise a flow-through flume system, based on the method of Peterson *et al.* (1983), has been selected because of its compact nature ($<1\,m^2$), low expense, and ease of installation and maintenance in high-discharge situations. This compact system is composed of a bank of Plexiglas cylinders attached to a flotation device that allows the apparatus to rise and fall with variations in stream discharge (Figure 32.2). Nutrients are dripped into upstream ends of the cylinders via Mariotte bottles. Banks of glass slides installed in downstream ends of cylinders (where nutrient concentrations are homogeneous), serve as substrata for algal colonization. Glass slides are retrieved through time to assess accrual of algal standing crop.

1. Construct a bioassay apparatus consisting of five 1.2 m sections of clear plastic tube of 9.2 cm diameter attached by U-bolts to the top of a sheet of Plexiglas (see Figure 32.2 for construction details). Each tube represents a treatment (e.g., tube 1, N enrichment; tube 2, P enrichment; tube 3, N+P enrichment; tubes 4 and 5, nonenriched controls). The apparatus is suspended from wood and styrofoam lateral floats. The upstream end of each tube should contain Lexan baffles to ensure turbulent mixing of the water and nutrient drip. A set of five microscope slide holders, each holding six slides attached to a strip of Plexiglas with rubber bands, is installed at the downstream end of each cylinder. Nutrients are introduced into the upstream end at a constant rate by siphoning concentrated solutions of $NaNO_3$ and KH_2PO_4 from 1-liter polyethylene Mariotte bottles through Teflon minibore tubing (0.56-mm-diameter) at a rate of $\sim$25 mL/h (see Chapter 8 for details on making and use of Mariotte bottle). In the experiments of Peterson *et al.* (1983), this resulted in soluble reactive phosphorus (SRP) levels 5–15 μg P/L and nitrate values 50–150 μg N/L above ambient stream water concentrations. Nutrient drip rates from Mariotte bottles should be calibrated daily. Water samples should be collected at the downstream ends of each tube every two to three days for analyses of NO_3 and PO_4.

2. Install the bioassay apparatus in an area of relatively uniform current velocity. The wood and styrofoam lateral floats should be adjusted so that they are submerged just below the water surface. The apparatus should be securely tethered to a rope extending across the stream. This installation method allows the apparatus to be relatively flood-tolerant. Additions of food coloring or Rhodamine-WT dye to the upstream ends of the tubes and timing of dye movement will indicate the rate of flow within the tubes. These should be compared to similar water movement measurements taken outside the tubes.

3. After one, two, and three weeks, retrieve a set of six slides from each tube and discard the two on either side. Each slide should be placed into a sealed plastic bag and carefully transported to the laboratory. Two slides from each tube can be used for chlorophyll *a* analyses and two slides for AFDM analyses. All algae should be carefully removed from both sides of each glass slide using a razor blade, rinsed into a beaker with distilled water, mixed with a magnetic stirrer, and subsampled for chlorophyll *a* and AFDM.
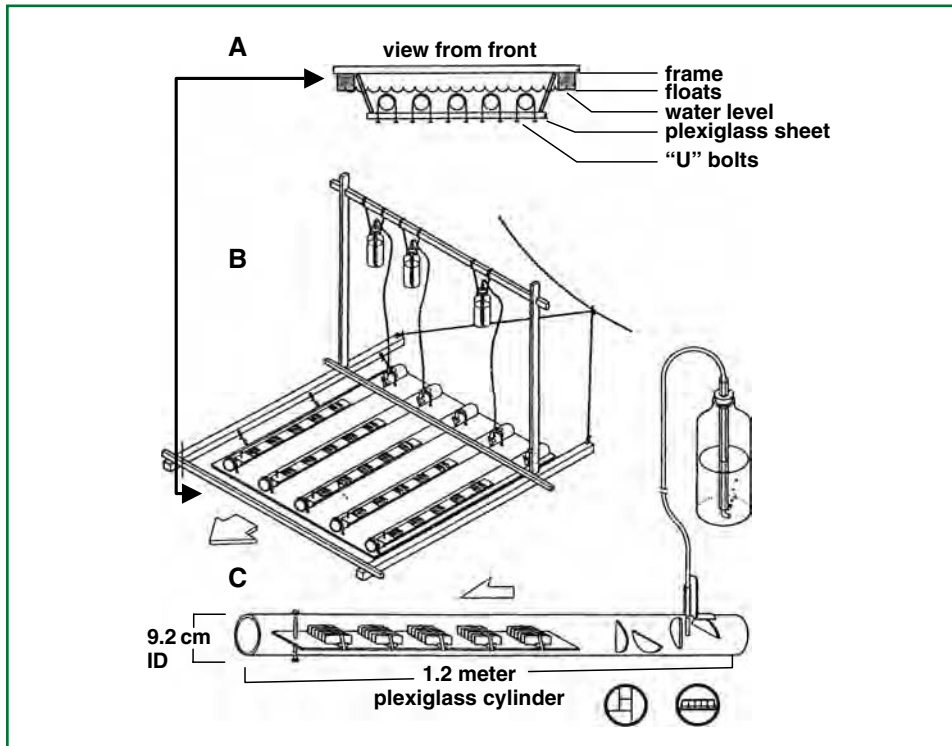
FIGURE 32.2 A. Cross-sectional diagram of continuous-flow periphyton bioassay system. Bioassay tubes were suspended beneath the surface from a pair of outrigger floats. B. Diagram of bioassay apparatus with Mariotte bottle frame tethered to a rope across the stream. Arrow denotes direction of water flow. C. Detailed drawing of a single bioassay tube showing the Mariotte bottle, mixing baffles, and slide-mounting apparatus (modified from Peterson *et al.* 1983).

C. Advanced Method 2: Nutrient Limitation of Algal Growth Using Whole-Stream Enrichment

In this method whole-stream nutrient enrichment will be employed by dripping concentrated $NaNO_3$ and/or KH_2PO_4 (or phosphoric acid) into the stream channel from a carboy fitted with a drip system. The decision regarding the nature of the nutrient addition will be determined by premeasurement of ambient nutrient levels (e.g., if the stream has low background phosphorus levels, then P enrichment may be selected; alternatively N enrichment may be desirable if ambient N levels indicate that the system is N-limited). Effects of whole-stream enrichment will be assessed by algal growth on artificial substrata (i.e., unglazed ceramic tiles) placed in both a pool and a riffle above and below the point of enrichment.

1. Use a large carboy (e.g., 50 liter), fitted with a spigot and vented by a narrow tube (to prevent changes in head pressure from affecting the flow rate) as a nutrient reservoir. Mount the carboy on a sturdy wooden stand or on the trunk of a riparian tree near a section of the stream where the channel narrows and is somewhat turbulent to enhance mixing of the added nutrients. Attach a length of tygon

tubing sufficient to reach from the spigot to the stream surface and culminating in a micropipet tip. The micropipet tip will drip nutrients from the carboy reservoir into the stream. Attach it to a ringstand for stability and regulate the rate of nutrient addition by adjusting the height of the ringstand relative to the carboy. Calculate the solute concentration needed to attain a desired stream concentration (see Appendix 32.1).

2. Using this example (Appendix 32.1), the nutrient reservoir must be replenished approximately every three days (72–80 h). Make sure to record the time that the injection is stopped and restarted, between carboys. The rate of nutrient input can be checked by recording the amount remaining in the carboy. The input rate should be calibrated daily by recording the time it takes to fill a volumetric flask (20–50 mL). Streamwater can be used to mix the reagents on-site. The flow rate with this type of apparatus will vary slightly as the liquid level in the carboy changes and also with changing viscosity due to temperature variations. The head variation can be minimized by mounting the carboy higher, thereby creating a large head and reducing the relative head variation caused by the difference in a full and a nearly empty carboy. If there are very large diel temperature shifts, however, some variation in flow rate will still occur.

3. Another technique for adding nutrients continuously to stream water utilizes a battery-operated continuous-flow pump (LMI High Efficiency DC Powered Metering Pump) that is powered using solar panels. The pump injects a concentrated nutrient solution upstream from a reservoir. The reservoir and pump setup needs to be placed in an accessible area with fairly open canopy for the solar panels to function.

4. As the injectate solution is being prepared, place 15 unglazed ceramic clay tiles randomly within both a riffle and a pool habitat, both above and below the source of nutrient enrichment (total of 60 clay tiles, with 30 upstream controls and 30 downstream enriched). Treatment tiles should be placed far enough downstream so that the injectate solution is well mixed with streamwater by the time it passes over the tiles. Mixing can be checked by injecting a few milliliters of Rhodamine WT (or other dye) at the drip site. Care should be taken to minimize the variability in current and depth between sites selected for tile placement within a given habitat so that tiles are exposed to a relatively narrow range of current velocities (e.g., 25–30 cm/s for riffle and 0–5 cm/s in pool) and depths.

5. Retrieve, from both the pool and the riffle habitat, five replicate clay tiles after one, two, and three weeks. Process and analyze algal periphyton for both chlorophyll *a* and AFDM as in the preceding exercises and in Chapter 17. Determine nutrient concentrations at control and treatment sites. Determine the mass of nutrient added from drip rates and residual solution.

D. Activities for Additional Study

Additional activities, involving more time and facilities include (1) running integrated bioassay experiments that combine two of the protocols presented above (e.g., nutrient-diffusing substrata and flow-through system) to experimentally separate effects of nutrients added from different sources and to evaluate algal response to nutrient perturbations at different scales (e.g., see Pringle 1987, 1990); (2) combining nutrient enrichment with ecosystem-level process studies (e.g., primary production and community respiration;

see Chapter 28); and (3) assessing algal community composition response to different nutrient treatments in each of the above methods (see Chapters 16 and 34).

Chlorophyll *a* and AFDM should be analyzed and graphed for each treatment as a function of time. Rates of algal standing crop accrual should be compared between treatments and/or habitats. Inferential statistics (e.g., analysis of variance) can can be used to examine differences among various treatments in specific experiments.

IV. QUESTIONS

1. How might the physical/chemical nature of nutrient-diffusing substrata interfere with nutrient treatment effects? Consider the following possible complications. For example, terracotta saucers are composed of clay, which is fine particles of hydrous aluminum silicates and other minerals that can act as a sink for certain elements such as phosphorus. Discuss the implications of this with respect to your experimental results [i.e., from the perspective of algal response to internal macronutrient and micronutrient stores (within nutrient-diffusing substratum) and to nutrients in overlying waters].

2. Contrast the effect of algal flora *on* ambient nutrient chemistry with respect to point-source nutrient amendments (nutrient diffusing substrata) and whole-stream nutrient enrichment. What effect would the relatively small biomass of algae on a phosphorus-diffusing substratum have on ambient nutrient chemistry versus the effect of algal flora within an entire stream reach enriched with phosphorus?

3. Why might algal response to nutrient-diffusing substrata not reflect water enrichment assays?

4. How might community composition and physiognomy (three-dimensional structure) of an algal community be affected by nutrients introduced from different sources (e.g., substratum versus water)?

5. Why must caution be exerted in extrapolating experimental results from one spatial scale to another?

6. Compare and contrast the advantages of different methods described above in terms of replicability of treatments. Which of the experimental designs employ true replication of treatments? Which experimental design(s) *is/are pseudoreplicated* (sensu Hurlbert 1984)? How might one avoid pseudoreplication?

7. How might grazing benthic invertebrates have affected your experimental results? Is there any evidence that grazing insects might have obscured measurable treatment effects in your experiment by reducing algal standing crop? How might you quantify this effect?

V. MATERIALS AND SUPPLIES

Laboratory Materials

> 0.45-μm Millipore filters (for filtration of water samples)
> 125-mL Polyethylene bottles (for collection of water samples)
> 5, 10, and 20 mL Pipets Acetone (for chlorophyll *a* analyses)
> Duco cement
> NaNO$_3$

KH$_2$PO$_4$
Polyethylene squeeze bottles (for distilled water washes of artificial substrata)
Whatman GF/F filters or equivalent (for chlorophyll *a* and AFDM samples)

Terracotta nutrient diffusing substrates

60 Clay flowerpot saucers (10.2 cm diameter)
Agar
Plexiglas 1/8″ thick (sufficient amount for 36 12 × 12-cm squares)
Wood for construction of installation "diamonds" for saucers

Flow-through flume system

Glass slides (for periphyton colonization)
Plexiglas cylinders and plates, U-bolts
Wood and styrofoam (for lateral floats)

Whole-stream enrichment

2 Carboys (50 liter)
20 Liter bucket
60 Unglazed ceramic clay tiles (e.g., 7.3 × 15.3 cm)
Large funnel
Tygon tubing Paddle (for stirring)
Pipet tip
Plastic spigot
Plastic tarp

Field Materials

Beakers (for algal homogenate)
Current velocity meter
Flagging
Meter sticks
Rhodamine WT dye or food coloring (Advanced Method 1)
Spherical densiometer
Toothbrushes and razor blades (for scraping algae)
Ziploc® (gallon size) for saucer or tile experiment retrieval
Ziploc® (sandwich bag size) for glass slide retrieval

Laboratory Equipment

Autoclave
Drying oven
Electronic balance (±0.1 mg)
Filtering apparatus
Magnetic stirrer and hot plate
Muffle furnace
Spectrophotometer or fluorometer
Vacuum pump

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Appendix 32.1 Calculations for Determining Solute Injection Rate for Specific Stream Concentration

The solute, its concentration in the injectate solution, and the volume of the carboy reservoir will vary among sites due to factors such as discharge, target concentrations above background, the drip rate that can be reliably sustained, and the length of the experiment. Consider the following example:

Stream discharge =	10 liters/s
Nutrient solute to be added =	$NaNO_3$
Target concentration =	$100\,\mu g$ N above ambient levels
Length of experiment =	14 days
Drip rate =	10 mL/min
Carboy size =	50 liters

1. Amount of water to be enriched (liters/hr): 10 liters/s $\times$ 60 s/min $\times$ 60 min/h = 36,000 liters/h
2. Amount of $NaNO_3$ amendment (g/h): 100 /xg N/liter $\times$ 36,000 liters/h/0.16 (proportion of $NaNO_3$ that is N) = 22,500,000 /Ag $NaNO_3$/h = 22.5 g $NaNO_3$/h
3. Drip Rate = 10 mL/min $\times$ 60 min = 600 mL/h; a 50-liter carboy will allow 50 liter/0.6 liter/h or 83 h of constant nutrient addition
4. Concentration of $NaNO_3$ in the carboy:
   22.5 g $NaNO_3$/h/0.6 liter = 37.5 g $NaNO_3$/liter
5. Mix into carboy 37.5 g $NaNO_3$/liter $\times$ 50 liter = 1875 g $NaNO_3$; fill to 50 liters

Tip. Place NaNO$_3$ in a separate container and add a known volume of water while stirring. Decant the dissolved nitrate solution into the carboy. Repeat until all NaNO$_3$ is dissolved. Then bring carboy up to 50 liters and stir. This will ensure that no nutrient salt remains in crystalline form in the carboy. Before mixing, cover the area below the carboy with a plastic tarp to contain accidental spills.

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Surface-Subsurface Interactions in Streams

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I. INTRODUCTION

Stream-riparian ecosystems are an amalgamation of several interconnected subsystems. In two dimensions, there are longitudinally arranged units of the wetted stream, subsystems created by variations in current within the wetted stream (e.g., edge and center subsystems, thalweg and backwaters), and lateral subsystems across the stream-riparian corridor defined by baseflow, annual floods (active channel zone), and vegetation type. A third dimension encompasses the *hyporheic* subsystem, which exists below the sediment surface of each of the longitudinal or lateral subsystems in most streams. The hyporheic zone is defined broadly as the region of saturated sediments and interstitial water directly beneath and lateral to the surface stream, which interacts via exchange of water and materials with the surface stream (see Chapter 6).

Adjacent vertical, lateral, and longitudinal subsystems interact via the movement of water and its load of dissolved and suspended materials. Although many dissolved materials behave as water does, suspended materials may be "filtered out" to varying extents by bed sediments. Thus, most particulate materials in transport may be left on the sediment surface where water enters the bed (*downwells*), while high oxygen and low dissolved nutrients characteristic of surface water are transported to the hyporheic zone. Conversely, subsurface waters may be low in oxygen but exhibit elevated concentrations of dissolved nutrients, which can supply nutrient-limited surface communities at sites of

subsurface discharge (*upwells*). Upwelling water usually represents a small input to a large flux (surface discharge), whereas downwelling water represents a large input to a smaller flux (hyporheic discharge). Organism movement across subsystem boundaries may also occur; for example, some benthic invertebrates seek refuge in the hyporheic zone during disturbances (spates or dry spells) (Palmer *et al.* 1992, Dole-Olivier *et al.* 1997, Gjerløv *et al.* 2003). Conversely, some insects reside in the hyporheic zone during their larval stage and may travel up to kilometers through the interstitial environment to emerge from the surface stream or river (Stanford and Ward 1988). Chapter 6 describes factors influencing the process of exchange between surface waters and the hyporheic zone. In this chapter, we focus on the consequences of this exchange for organisms and ecosystem processes.

If we take a subsystem approach to studying stream ecosystems, then we can describe the properties of each component, its linkage with other subsystems (i.e., what kinds of transfers take place), and, perhaps most interesting, the *consequences* of such interactions for biotic communities in each subsystem. Hydrologists, fish biologists, and stream ecologists long have known that surface and subsurface waters interact (e.g., Wickett 1954, Vaux 1968, Grimm and Fisher 1984), but a burst of research over the last two decades has led to increased understanding of the ecological significance of groundwater-stream water interactions (Hynes 1983, Gibert *et al.* 1994, Brunke and Gonser 1997, Boulton *et al.* 1998, Jones and Mulholland 2000).

Studies of the interaction of surface and subsurface subsystems can improve understanding of organism distribution, the composition of communities, and the processes of ecosystem metabolism and nutrient dynamics (Figure 33.1). For example, in Sycamore Creek, Arizona, nitrogen is the limiting nutrient for primary production (Grimm and Fisher 1986). Hyporheic water that is enriched in nitrate because of nitrification in hyporheic sediments enters the surface stream at discrete upwelling zones (Valett *et al.* 1990, Jones *et al.* 1995a), which increases nitrate in the surface water at reach, channel unit, and subunit scales (Dent *et al.* 2001). At these sites algal biomass is higher (Henry and Fisher 2003), communities are dominated by filamentous green algae, and algae recover faster after disturbance than at downwelling sites (Valett *et al.* 1994). In Sycamore Creek,
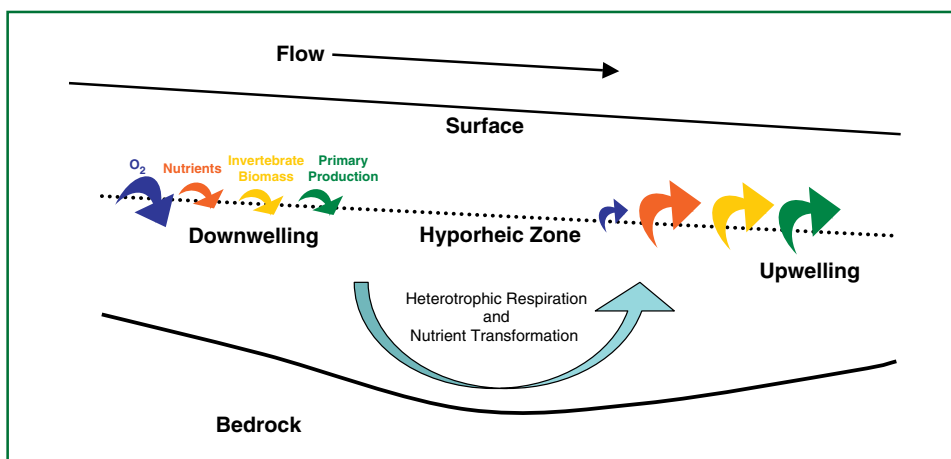
FIGURE 33.1 A conceptual model of some effects of hyporheic exchange on surface biota and ecosystem processes in Sycamore Creek, Arizona. (Adapted from Jones *et al.* 1995a.)

uptake by algae (Grimm 1987) and riparian vegetation (Schade *et al.* 2005) results in longitudinal depletion of nitrate; downstream algal communities have lower biomass and recover more slowly following disturbance (Valett *et al.* 1994) and often are dominated by nitrogen-fixing cyanobacteria (Fisher *et al.* 1982, Henry and Fisher 2003). A parallel consequence of surface-subsurface interaction for the hyporheic biota also has been discovered in Sycamore Creek. High dissolved oxygen and labile organic carbon in surface water are supplied to hyporheic microbial communities, which show higher respiration rates at downwelling zones than at upwelling zones (Jones *et al.* 1995b). Moreover, studies have described how "hotspots" of metabolic activity and nutrient processing occur at multiple spatial and temporal scales along flow-paths within the hyporheic subsystem (Holmes *et al.* 1994, Schade *et al.* 2001, McClain *et al.* 2003).

The objectives of the methods in this chapter are to describe the physical, chemical, and biological properties of surface and subsurface environments at sites of exchange. This description will permit inference regarding the effect of surface-subsurface interactions on the distribution of biota (see basic methods below) and ecosystem metabolism (see advanced methods below). A summary of the techniques for measuring hyporheic exchange is found in Chapter 6, and methods for estimating metabolism are described in Chapter 28. Methodological challenges may differ between environments (e.g., small streams and large rivers). Moreover, hyporheic interaction occurs across a hierarchy of scales (Stanford and Ward 1993, Fisher *et al.* 1998, Baxter and Hauer 2000, Woessner 2000, Dent *et al.* 2001), and approaches may vary depending on the scale(s) of interest. The techniques we present here are designed for small streams and focus on patterns that may occur within a stream reach. In some cases, we provide a range of procedural options from which to choose, depending on the resources and equipment available and the depth and focus of the study.

II. GENERAL DESIGN

The basic design of the methods described here is a comparison of properties of surface and subsurface environments at points of exchange (upwelling and downwelling zones). An overall system map provides a context for the comparisons. The extent of replication is dependent upon time constraints; although the techniques described are relatively simple to perform, they may be time-consuming. In an educational context, a relatively simple, descriptive design may be chosen where one upwelling and one downwelling zone are compared; however, a more rigorous study would replicate (n = 3, at least) the hydrologic exchange zones. In the "Basic Methods" section we describe mapping the channel morphology, measuring hyporheic exchange, and sampling surface and subsurface chemistry and biota that may be influenced by groundwater-surface water interactions. In the "Advanced Methods" section we describe measurement of surface metabolism and hyporheic zone respiration, with the goals of measuring the contribution of the hyporheic zone to ecosystem metabolism and comparing this process in upwelling and downwelling areas.

A. Site Selection

The studies described in this chapter are designed for small streams (typically first to fourth order) with beds dominated by fine- to medium-grained sand or gravel sediments of high hydraulic conductivity at low or baseflow conditions. Difficulties in obtaining

interstitial samples from very fine silt or clay sediments and installing wells or mini-piezometers in coarse sediments often preclude application of these techniques to such systems, though "heavy-duty" piezometer designs have been developed to cope with the latter (see Baxter *et al.* 2003 and Chapter 6). In the optimum stream for these studies, it should be possible to move sediments around with a shovel or insert minipiezometers or other sampling devices with a sledge hammer and steel rods. A single reach or two reaches (20–100 m length) in close proximity should be selected for study. Ideally, the stream selected should be one in which some measures of hyporheic exchange (such as coarse-scale measures of hydraulic gradients and flow accretion) had been conducted, as the net exchange character of the reach (i.e., gaining, losing, or through-flow; Woessner 2000) sets the context within which more localized processes of exchange occur (Baxter and Hauer 2000, Dent *et al.* 2001). Depending on the educational or research goals, an open canopy permitting growth of abundant algae or macrophytes, presence of a beaver dam, or distinct fish nests (called redds) may be other desirable (but not required) site characteristics (e.g., White 1990).

B. Overview: Basic Methods

Here we describe techniques for measuring hyporheic exchange and for sampling surface and subsurface chemistry and biota that may be influenced by this exchange. An overall map of the reach should be constructed, on which hydrological, chemical, and biological data can be plotted. This may be most effective if combined with methods described in earlier chapters (e.g., Chapters 2 and 5). During mapping, locate and mark likely upwelling and downwelling zones, based on bedform variations (Figure 33.2A), and measure stream gradient and discharge. A spatially explicit description of morphometric parameters such as stream width and depth, stream gradient, active-channel width, riparian zone(s) width, and location of geologic or structural features (e.g., bedrock outcrops, rocky riffles, or woody debris accumulations) will help in understanding variables that influence subsystem exchange and its consequences for the biota.

To estimate the potential for exchange between hyporheic and surface waters *vertical hydraulic gradient* (VHG) must be measured. This is a measure of the pressure difference between surface and subsurface waters; its sign indicates whether water is entering (negative VHG; *downwelling*) or discharging from (positive VHG; *upwelling*) the hyporheic zone. Vertical hydraulic gradient can be measured using a *minipiezometer* (Lee and Cherry 1978, Baxter *et al.* 2003) and *hydraulic potentiomanometer* (Winter *et al.* 1988). The minipiezometer is inserted to a standard depth (L) in the sediments using a T-bar or one of the other techniques described in Chapter 6. Water is then drawn from the piezometer and the surface water through flexible tubing to the hydraulic potentiomanometer, where differences in height between subsurface and surface water levels (Δh) can be more easily read (Figure 33.2B). Vertical hydraulic gradient is calculated as:

$$VHG = \frac{\Delta h}{L} \tag{33.1}$$

Alternatives to using a potentiomanometer for measuring water levels are also described in Chapter 6. While measurement of VHG using this method is simple and provides useful
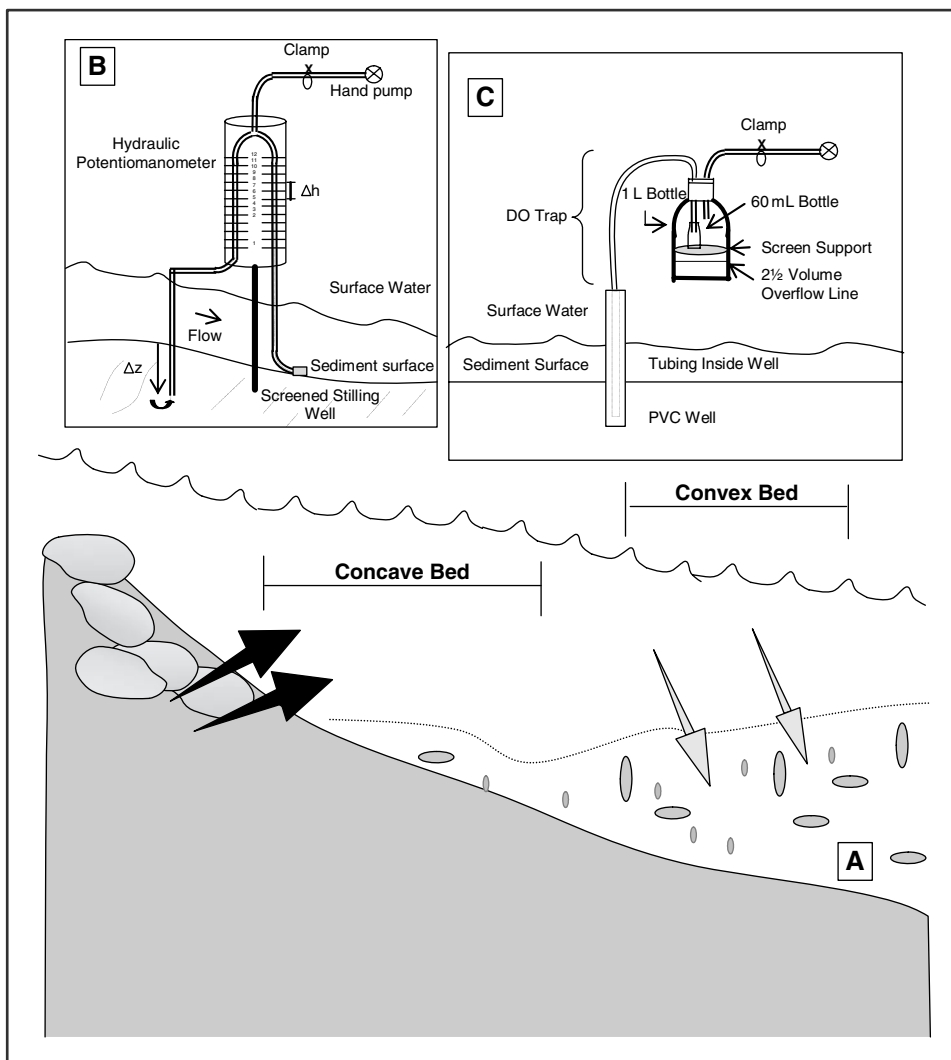
FIGURE 33.2 Methods and terms used in the study of surface-subsurface exchange. A. Illustration of the base of a riffle and head of a sand/gravel run, showing position of upwelling zone (in a concave bed site) and downwelling zone (in a convex bed site). B. Minipiezometer and hydraulic potentiomanometer installed at an upwelling site. Note positive Δh, denoting upwelling. C. Sampling well and DO trap installed at a downwelling site.

information concerning exchange, inference that exchange is actually occurring must be made with caution. Subsurface water may be at pressure but prevented from upwelling by low permeability sediments (Freeze and Cherry 1979, Schalchli 1992). Similarly, algal mats or other material (e.g., leaves and other detritus) may clog sediments and obstruct penetration of the bed (Kuznetsov 1968). Characterization of actual subsurface flow rates requires measurement of both VHG and hydraulic conductivity (K; see Chapter 6). In addition to measurement of VHG, falling head tests or dye injections may be used to

determine subsurface flow velocity, and the former may also be used to locate sites of lateral exchange (Chapter 6).

After delineating local upwelling and downwelling zones using measures of bedform and VHG, chemical and biological sampling can be conducted to identify potential surface and subsurface patterns associated with hyporheic exchange. Identify and map locations of major patches of algae, macrophytes, and sessile invertebrates, as well as features such as fish redds or beaver dams in the stream reach. Nondestructive methods of counting and measuring patch size of organisms should be used where possible. Collect benthic core samples or small cobbles at each upwelling and downwelling zone for measurement of chlorophyll *a* and algal biomass, determination of major algal types (e.g., nitrogen fixers vs. nonfixers; Henry and Fisher 2003), and quantification of benthic invertebrate numbers, community structure, or biomass (e.g., Pepin and Hauer 2002). If applicable, measure hydrologic characteristics (i.e., VHG, K) associated with major algal or macrophyte patches, fish redds, or beaver dams.

Wells should also be installed within upwelling and downwelling zones so that water samples can be collected for determination of temperature and dissolved oxygen of surface and subsurface water. Although water samples may be withdrawn through the mini-piezometers, it is often easier to sample subsurface water from wells. Wells (either open ended or slotted) are most simply constructed from lengths of PVC pipe; a heavy-walled (schedule 40) PVC with an internal diameter of 15–20 mm has been used successfully (see Chapter 6). Wells are fitted over a steel T-bar that is slightly longer than the well and inserted by pounding the T-bar into the bed to a depth marked on the well. The T-bar is then carefully withdrawn, leaving the well in place. Temperature is measured using a thermometer suspended on a thin line into the well. Dissolved oxygen may be measured either by the Winkler method (Chapter 5) or with an oxygen meter if a large-diameter well is used (see Chapters 5 and 6). A simple pump and trap apparatus can be made to collect water samples for dissolved oxygen analysis (Figure 33.2C).

If wells are installed, hyporheic invertebrates can also be sampled, allowing subsurface and surface invertebrates to be compared at upwelling and downwelling locations. Since a large volume of water from wells is required to collect sufficient numbers of hyporheic invertebrates, a small diaphragm-pump is used and water (for chemical analysis) is collected after sieving for invertebrates. This method samples invertebrates that are loosely associated with sediments or are swimming through interstitial space, and tends to underestimate more sessile or sedentary forms (for details, see Fraser and Williams 1998, Scarsbrook and Halliday 2002, Hahn 2003). For the exercise described here, contrasts between sites using comparable methods are of primary interest. Numbers and kinds of hyporheic invertebrates (see Merritt and Cummins 1996, Thorp and Covich 2001) might be expected to differ between upwelling and downwelling zones, because of differential species' tolerance of oxygen, temperature, and other factors. Differences between chemistry of hyporheic and surface waters in the two zones may also be used to interpret differences in biota.

C. Overview: Advanced Methods

Here we describe two approaches for (1) estimating the contribution of the hyporheic zone to overall ecosystem metabolism and (2) making a comparison of surface metabolism (net production and respiration) and subsurface respiration at upwelling and downwelling locations. For many years, stream ecologists' principal tool for measuring metabolism was a chamber in which streamed materials were incubated and changes in dissolved

oxygen concentration in both dark and light were measured (see Chapter 28). Whereas the chamber technique provides an important measure of control, it may also suffer from nonrepresentative sampling, disruption of the enclosed community, and the generally artificial environment that is created (Pusch and Schwoerbel 1994, Naegli and Uehlinger 1997). In contrast, the open-channel or whole-stream method of measuring metabolism (first introduced by Odum 1956) may provide more realistic measures of metabolism and has recently been improved to increase its accuracy, precision, and ease of use (Hall and Tank 2005). In particular, better estimates of reaeration rates can now be obtained using injections of tracer gases (Wanninkhof *et al.* 1990, Marzolf *et al.* 1994, Young and Huryn 1998) and automated, continuously recording oxygen meters are both precise and affordable. Though the principles behind measuring surface metabolism and subsurface respiration rates are relatively straightforward, in practice these techniques require a wide array of measurements and careful attention to detail, and both the methods and calculations continue to be modified and improved (for more details, see Fellows *et al.* 2001, Hall and Tank 2005; Chapter 28).

Ecologists have shown that the hyporheic zone can play a significant role in overall system metabolism (Grimm and Fisher 1984, Pusch and Schwoerbel 1994, Naegli and Uehlinger 1997, Fellows *et al.* 2001), and with increased use of the open-channel technique has come recognition that metabolism estimates may be substantially influenced by inputs of low-O_2 water from the subsurface (McCutchan *et al.* 2002, Hall and Tank 2005). However, as pointed out by Hall and Tank (2005), upwelling hyporheic water and groundwater from deeper sources may have distinct oxygen concentrations, making it necessary to locate where new water enters the stream reach and measure its oxygen concentration at that point. If the goal is to simplify the exercise outlined below for educational purposes, we recommend studying a section of stream where hyporheic exchange occurs but where deep groundwater springs are not present.

Following the general approach of Fellows *et al.* (2001), we outline techniques for estimating the contribution of the hyporheic zone to ecosystem metabolism and for comparing sub-reach locations of upwelling and downwelling. The approach in the first case uses a combination of both chamber and open-channel measurements. An advantage of open-channel methods is that they integrate contributions from patches of different metabolic activity throughout a stream reach (Naegli and Uehlinger 1997), whereas chamber techniques may be more appropriate at finer spatial resolution for delineating difference among those patches. The second approach we present therefore relies on comparisons of patch-specific metabolism measured with chambers.

To evaluate the relative role of the hyporheic zone in whole-system metabolism, we can use the difference between estimates of open-channel respiration and benthic respiration (measured via chamber method) as an estimate of respiration occurring in the hyporheic zone (Grimm and Fisher 1984, Naegli and Uehlinger 1997, Fellows *et al.* 2001). Using the two-station diel method, the open-channel technique provides an integrated estimate of metabolism for an entire reach, and involves application of a mass-balance approach to samples of dissolved oxygen taken at upstream and downstream locations over a 36–48-hr period (Chapter 28). Measurements of benthic metabolism may be obtained by incubation of streambed sediments in chambers. Sediments should be collected from upwelling and downwelling locations within the reach, and their metabolic activity can be compared. Subsequently, these estimates may be averaged (or weighted by the spatial extent of upwelling and downwelling areas) to obtain an estimate of benthic respiration over the entire reach. Making the assumption that respiration in the water column is negligible relative to benthic and hyporheic zone contributions, the difference between

the open-channel respiration estimates and the benthic respiration estimates may be used to approximate the hyporheic zone's contribution to metabolism for the whole stream reach.

Though open-channel measurements could be attempted at localized upwelling and downwelling patches (using the single-station technique, Chapter 28), this method may not work as well for gathering information about metabolic activity at such high spatial resolution. Rather, this is one of the strengths of the chamber method, because sediments from specific locations are removed and incubated in isolation from the potentially confounding effects of the rest of the system. Therefore, to compare upwelling and downwelling subsystems, surface and subsurface sediments are incubated in chambers, allowing comparison of benthic and hyporheic respiration in localized upwelling and downwelling patches within the reach. Direct estimates of subsurface respiration by incubating hyporheic sediments in a recirculating chamber or microcosm (see Pusch and Schwoerbel 1994, Baker *et al.* 2000, Fellows *et al.* 2001, Crenshaw *et al.* 2002), and surface respiration is measured as just described. In addition, integrating the chamber-derived hyporheic respiration estimates over the entire stream reach will yield an estimate that can be compared to that obtained using the approach outlined above. These are the two techniques we compare here, though recently other micro- and mesocosm designs have also been employed (e.g., Findlay *et al.* 2003, Marshall and Hall 2004).

III. SPECIFIC METHODS

A. Basic Method 1: VHG and Distributions of Biota

Field Protocol: Creating a Base Map

The detail of the map, and the tools applied, will depend upon the goals of the research. Basic mapping can be performed using a tape measure and hand level (or an inclinometer) and can be drawn on grid paper (see below). More detailed maps will require use of advanced tools (such as a laser theodolite) to survey points throughout the reach, and mapping software (such as Surfer®) to map channel shape, along with the locations of minipiezometers, water table elevations, and the locations or values of other chemical or biological factors of interest (for examples, see Valett *et al.* 1994, Baxter and Hauer 2000).

1. Run a meter tape along the length of the reach and flag at evenly spaced intervals. Depending on size of the reach, flagging should delimit transects that will be 4–10 m apart (aim for 5–7 transects per reach).
2. In the subreaches between each transect, roughly map out bedform variations, looking for likely upwelling (concave) or downwelling (convex) sites (Figure 33.2A). Where these transitions are found, mark them for use in determining locations for measurements of surface-subsurface exchange.
3. At each transect, measure and record stream width, active channel width, and width of riparian zone. Measure depth at evenly-spaced intervals across the stream. Measure stream velocity at these same intervals (see Chapters 3 and 4).
4. Map substratum type: in the subreaches between transects, record major substratum type. Record location of any variations in substratum that occur between transects (e.g., a short riffle dominated by cobble within a 20-m subreach

dominated by sand). These should be accurately located on the map so that comparison can be made with upwelling/downwelling location.

5. Fill out the map: map location and size of other physical features.

Field Protocol: Hydrologic Measurements

1. Set up a data sheet as in Table 33.1.
2. Beginning at the top of the reach, install minipiezometers and measure VHG at midchannel (or nearer the banks in swiftly flowing water) at evenly spaced intervals. Based on studies of other small streams (e.g., Valett *et al.* 1994, Baxter and Hauer 2000), approximately 30–50 measurements per reach may be adequate to characterize exchange patterns. However, the number of samples required will depend on the spatial heterogeneity of the study system and the questions being asked. Once minipiezometers are installed and measurements of VHG are taken (see following), plot the locations of these measurements on the base map.

   a. At each measurement point, use a small sledge hammer to insert a T-bar fitted with a slightly shorter well marked at 25 or 30 cm into the sediment. Carefully withdraw the T-bar and drop a minipiezometer into the well. Push the minipiezometer to the bottom of the well using the T-bar, and, holding the T-bar in place, carefully withdraw the well, leaving the minipiezometer in the stream bed.

   b. Attach the tubing from the hydraulic potentiomanometer to the minipiezometer, and set the tubing with the stilling well on the stream bed (out of direct current). Slowly suck water from both ends into the hydraulic potentiomanometer, being careful to avoid air bubbles. When there is a continuous column of water in both tubes, clamp the tubing above the Y. Slowly release the clamp, allowing the water levels to drop down to the measurement scale.

   c. Read and record the potential difference (*hydraulic head*; Δh). Be sure to record the *sign*: if the hyporheic column is higher, the sign is +; if the surface column is higher, the sign is − (see also Figure 33.2B). Also record the depth of the piezometer (ΔL). Enter these values in the table next to the value for distance along reach. Record any other information on location.

TABLE 33.1 **Example Data Sheet for Hydrologic Parameters.**

Distance along Reach	Δh (cm)	Δz (cm)	Notes
0 m	+2.5	25	base of riffle
5 m	-0.5	25	right bank higher elevation
5 m	+1.0	25	midchannel

sample data →

3. When a VHG measurement indicates a possible strong upwelling or downwelling zone, note that location with flagging on wire stakes for chemical and biological sampling.

4. At sites where the stream is braided or appears to vary laterally in elevation, flow velocity, or substratum, make measurements of VHG at additional lateral locations.

5. After VHG has been measured for the entire reach, return to a site of upwelling for measurement of interstitial flow rate. One simple method is to use a dye injection. Inject a small bolus of dye (fluorescein is highly visible and thus useful for such qualitative sampling) 5 cm beneath the sediment surface either with a syringe fitted with a long cannula or through a minipiezometer. Record injection time. Wait for dye to appear at the sediment surface; record time of first appearance. Measure distance from injection point to emergence point and calculate distance traveled by the dye. Repeat at another site to verify rate. If dye does not appear, dig at the point of injection to locate dye bolus. If dye cannot be found, the site is not an upwelling site!

6. You may also use minipiezometers and dye injections to identify sites where water enters the stream from lateral interstitial flow (parafluvial zone).

Field Protocol: Mapping and Sampling of Biota

1. Walk along the reach and identify major patch types of algae, macrophytes, and/or sessile invertebrates, as well as features such as beaver dams or fish redds. In a field course situation, if students don't know the names of organisms, have them give each type a code name (e.g., green alga 1; invertebrate 3, etc.).[1] Record their locations on the reach base map.

2. Draw major patches on the map. Measure the variables found on Table 33.2 (select those that apply to study system from the following list) and record them on a data sheet.

3. Collect a benthic invertebrate core sample at each upwelling and downwelling zone selected for sampling (see Chapters 19 and 20 for additional ideas on sampling invertebrates).
   a. Core to standard depth (5–10 cm) using a section of PVC pipe (5–10 cm diameter), and place core contents into a small bucket.
   b. Add water; elutriate sample by swirling bucket and pouring off water plus invertebrates (plus detritus and algae) through a 62-μm mesh sieve or net. Repeat at least 5–7 times (or until no additional invertebrates are collected).
   c. Rinse contents of sieve/net into a labeled bag or vial with 70% ethanol (final concentration) to preserve.
   d. Replicate benthic invertebrate cores if sites (upwelling, downwelling) are not replicated (n = 3 minimum).

4. Collect an algal core sample at each upwelling and downwelling zone selected for sampling (see Chapters 16 and 17 for additional information on sampling algae).

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[1] Focus on the most abundant or easily recognized patch types.

TABLE 33.2	Biotic Variables to Be Measured and Recorded.	
Patch Type	Patch Characteristics to Measure	Location (Reference to Detailed Maps)
Algae — mats or filaments	Mat size (length × width)	edge or center
	Algal height or mat thickness	upwelling or downwelling
	Algal condition	distance along reach
Algae — microscopic communities on sediments	Color	major substratum types
	Continuity of cover	major physical features
		(these notes should be taken to aid in placing patches on map)
Macrophytes	Bed Size (length × width)	
	Plant height	
	Plant condition	
	Plant density (low, medium, high	
Invertebrates – large sessile (attached) or conspicuous grazers	Density (quantitative; number per unit area)	
	Diversity (number of taxa)	
	Apparent feeding mode (see Chapter 21)	

    a. Core to standard depth (2–3 cm) using a 3–6 cm diameter section of tubing, or a cut-off syringe.
    b. Drain water from core without losing sample.
    c. Place sample into a labeled bag and store on ice.
    d. Replicate algal cores if sites are not replicated.
5. Alternative to coring: If substrata are too large for coring, collect cobbles for determination of algal biomass and species composition. Place 2–3 cobbles from each site into a labeled bag, and store on ice.
6. Measure hydrologic characteristics of particularly striking biotic patches, if not located in sampling sites. For example, algal mats or macrophyte beds may be especially abundant at points where lateral interstitial flow enters the stream, or at the heads of small side channels formed where water upwells from the sediments. In addition, features such as beaver dams, macrophyte beds, or fish redds may create local patterns surface-subsurface exchange (White 1990). As before, record relevant patch characteristics as well as hydrologic characteristics (VHG, flow direction determined using dye injections).

Laboratory Protocol

1. Calculate *VHG* $(= \Delta h/\Delta L)$.
2. Calculate interstitial flow velocity (distance/time).
3. Place VHG, flow path direction (if any), and biotic patch data on the reach base-map.
4. Process algal core samples.
   a. Obtain a small "grab sample" (a few bits of gravel or mL of slurry) and place in a vial with algal preservative (see Chapter 16) for later identification of species.
   b. Divide the gravel/sand sample in half and analyze subsamples for ash-free dry mass (AFDM) and chlorophyll *a* (use a modification of protocols in Chapter 17) wherein subsamples are extracted (chlorophyll *a*) or dried and ashed (AFDM) directly).
   c. Calculations: use area of corer to express chlorophyll *a* and AFDM on an areal basis; multiply by 2 to correct for subsampling.
5. Process algal cobble samples.
   a. Scrape and brush material from cobbles into a small volume of distilled water. Add water to this to make up to standard volume (e.g., 50 or 100 mL).
   b. Homogenize the scraped material plus water.
   c. Subsample by pipetting a known volume of the homogenate.
   d. Filter the chlorophyll *a* subsample and analyze filters for chlorophyll *a*. Place AFDM subsample into drying vessel. Preserve a third subsample for algal identification. Measure the area of the cobbles (see protocols in Chapter 17).
   e. Calculations: use cobble area to express chlorophyll *a* and AFDM on an areal basis; multiply by total homogenate volume/subsample volume to correct for subsampling.
6. Identify and count invertebrates in preserved samples (see protocol in Chapter 20).

B. Basic Method 2: Temperature, Chemistry, and Hyporheic Invertebrates

Field Protocol: Measuring Temperature and Dissolved Oxygen

1. Install wells at upwelling and downwelling sites selected for sampling.
   a. Insert a 50-cm T-bar fitted with a 48-cm well to a sediment depth of 30 cm using a sledge hammer.[2]
   b. Carefully remove the T-bar while holding the well, leaving the well in place.
   c. Bail the well: insert long tubing connected to dissolved oxygen (DO) trap into well (see Figure 33.2C). Push tubing to the bottom of the well, and then pull up slightly (1 cm) to avoid aspirating sand. Pump ca. 300 mL water from the well and discard.[3]
2. Collect hyporheic oxygen samples.
   a. Insert a DO bottle (with stopper removed) into the trap. Replace and tighten top of trap, ensuring that long tubing piece inside the cap is inserted into the DO bottle.
   b. Pump water from the well to overflow the DO bottle 2.5 times.

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[2] Top of well must be above water surface level, so use a longer T-bar and well in deeper water.
[3] Temperature can be measured on this water.

    c. Important: clamp the tubing to stop flow of water from the well, and then remove the DO bottle.

    d. Fix sample using Winkler reagents (see Chapter 5), and store in the dark.

    e. Alternatives (if difficulty in obtaining 60-mL sample is encountered): (1) Pump a sample of hyporheic water into an empty bottle or jar and measure its oxygen content using a dissolved oxygen electrode (see Chapter 5); (2) drop electrode into wide-bore well (see Chapter 6); (3) fill a 10-mL syringe from a continuous column of water pumped from the hyporheic zone; analyze dissolved oxygen using a micro-Winkler technique (see Grimm 1987 or Chapter 5).

3. Measure and record hyporheic water temperature. Suspend thermometer into well, withdraw and read temperature, or read directly from DO probe (wide-bore wells).

4. Collect surface oxygen samples using the DO trap employed in (2), pumping from the surface stream. Fix and store in dark. Alternative: use DO electrode.

5. Measure and record surface water temperature.

Field Protocol: Sampling Nutrient Chemistry and Hyporheic Invertebrates

1. Obtain samples of surface water at locations where well have been installed. First rinse the bottle(s) 3 times with stream water, then fill from the thalweg.

2. After oxygen samples have been obtained from the well, collect hyporheic water and invertebrate samples.

    a. Insert long end of tubing connected to the diaphragm pump setup into the well. Push tubing to the bottom of the well, and then pull up slightly (1 cm) to avoid aspirating sand.

    b. Ensure that cap is tightened onto the collection bottle, and then begin pumping slowly. Diaphragm pumps can generate quite a vacuum, so be sure you do not pump so fast as to empty the well or aspirate stones. Pump until 2, 3, or 4 L of water is collected.[4]

    c. Remove bottle cap and slowly pour contents *through* a 62-μm mesh net *into* (1) rinse bottle (for rinsing invertebrate net) and (2) water sample bottles, first rinsing bottles and then filling.

    d. Rinse net contents into a prelabeled ziploc® or jar, using the filtered water in the rinse bottle. Store on ice.[5]

Laboratory Protocol

1. Acidify and titrate oxygen samples (see Chapter 5).

2. Filter water samples and analyze for nutrients (nitrate-N, ammonium-N, soluble reactive P, others as suggested by prior data or coordinator) using standard methods (APHA 1995).

3. Identify and count hyporheic invertebrates in samples from wells. Since many species of hyporheic invertebrates are poorly known, the level of taxonomic

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[4] In finer sediments, volumes may have to be reduced.

[5] If samples cannot be processed within 2–3 d, rinse net contents into Ziploc® or jar with 70% ethanol to preserve the sample. It is best, however, to examine specimens live if at all possible.

resolution may be relatively coarse. In a field class situation, however, encourage students to recognize abundant species (even if they don't know their names) to determine whether differences in species composition exist between upwelling and downwelling zones. See Chapter 19 for protocols on identifying meiofaunal invertebrates.

C. Advanced Method 1: Measuring Surface Metabolism and Subsurface Respiration

Field Protocol

1. Use the open-channel, two-station diel method to obtain an integrated measure of ecosystem metabolism for the study reach (see Chapter 28 for details).
   a. Open-channel metabolism is measured by monitoring dissolved oxygen concentrations at upstream and downstream locations over a 36–48 hr period. If available, place an automated, continuously recording DO meter at the upstream and downstream ends of the reach, and record DO concentrations at 15-minute intervals. If a recording probe is not available, collect water samples for analysis of DO every 30 min or hour for the duration of the study.
   b. To account for exchange of oxygen between the stream and atmosphere, a reaeration coefficient must be calculated. This calculation can be performed based on measurements of mean velocity (V/cm/s) and mean depth for the reach (H, in cm). An alternative method involves coinjection of a conservative tracer (chloride or bromide) and a volatile gas tracer (propane or sulfur hexafluoride [SF_6]) at a constant rate and concentration, followed by determination of the longitudinal decrease in steady-state concentrations (Wanninkhof *et al.* 1990, Marzolf *et al.* 1994). For more detail and other techniques, see Chapter 28. If there are nonhyporheic groundwater springs in the reach, their volume and oxygen concentrations must also be accounted for, and approaches to this problem are addressed in Chapter 28 (but see also Young and Huryn 1998, McCutchan *et al.* 2002, Hall and Tank 2005).
2. Measure benthic metabolism and hyporheic respiration at upwelling and downwelling locations using the chamber technique.
   a. For measurement of benthic metabolism, chambers as simple as Ziploc® or stoppered cores, or chambers as sophisticated as those described in Chapter 28, may be used. However, to measure subsurface respiration directly, hyporheic sediments should be incubated in recirculating chambers in the lab (see Pusch and Schwoerbel 1994, Baker *et al.* 2000, Fellows *et al.* 2001, Crenshaw *et al.* 2002). The problems, assumptions, and calculations associated with use of such chambers are described in Chapter 28 and in the literature cited previously.
   b. Collect material to be incubated and water for incubations. For surface and hyporheic incubations, collect material within the top 5 cm and 10–30 cm depth, respectively. For especially fine benthic sediments, it may be necessary to place trays filled with precleaned sediments in the stream for colonization by periphyton several weeks before measurements are made. Label chamber with hydrologic type (up- or downwelling), subsystem (surface or hyporheic), and replicate number (suggested minimum number of replicates = 5; giving total N = 5 × 4 treatments = 20).
   c. Collect a reservoir of "initial" stream water from each of four sites: surface/upwelling; hyporheic/upwelling; surface/downwelling; hyporheic/downwelling.

Use wells to obtain hyporheic water. After thoroughly mixing each of the four reservoirs of initial stream water, collect triplicate dissolved oxygen samples from each using a hand pump-DO trap apparatus (see Basic Methods above) *or* insert dissolved oxygen electrode into reservoir and measure *initial* dissolved oxygen.

d. Carefully fill chambers with water from the appropriate reservoir and add material to be incubated.

e. Incubate chambers in light (surface) or dark (bury in sediments or cover with black plastic) *under stream water* (to maintain realistic temperature) for 1–1.5 hr.

f. Carefully measure "final" dissolved oxygen (dissolved oxygen meter or Winkler method).

g. Return contents of chambers to laboratory for analyses of chlorophyll *a* and AFDM.

Laboratory Protocol

1. Obtain estimate of whole-stream metabolism using the open-channel technique.
   a. Titrate dissolved oxygen, if required.
   b. Calculate Gross Primary Production (GPP) and Ecosystem Respiration (ER). GPP is the sum of the net production of O_2 during the light period and the mean hourly dark respiration rate multiplied by the length of the light period. ER is calculated as the mean hourly net production rate of O_2 during the dark period extrapolated to 24 hr. Calculate respiration rates during the light period using the average respiration rates of the nights before and after the day of interest. For more details of calculations, see Chapter 28.
   c. Calculate aerial rates of GPP and ER by dividing by the bed surface area of the reach. Scale the value to a 24-hour period for ease of comparison.

2. Measure respiration of hyporheic sediments using recirculating chambers.
   a. Recirculation in such chambers prevents water from becoming stagnant, which can confound measures of oxygen change. However, this method requires removing sediment from the field and processing in the lab. Microcosms can be constructed out of Plexiglas tubes (easier to see bubbles that will need to be removed) or PVC pipes (7 cm diameter works well). To prevent preferential flow along the inner walls, roughen with 100-grit sandpaper. (For more details about these chambers, see Pusch and Schwoerbel 1994, Baker *et al.* 2000, Crenshaw *et al.* 2002.)
   b. Follow steps a–c outlined in field protocol above.
   c. Carefully fill chambers with water from the appropriate reservoir and add material to be incubated. Try to remove all bubbles. Cap the microcosm on either end with caps that have been drilled and fitted with tubing. Water should flow vertically from the bottom of the core to the top at a known rate (dependent on measured subsurface flow rates at the site) using a peristaltic pump and recycled for 3–6 hr.
   d. Incubate chambers in dark (covered with black plastic) and try to maintain realistic stream or hyporheic water temperature for 3–6 hr.
   e. Carefully measure dissolved oxygen (Winkler method) ∼ every 1 hr to calculate the rate of change. To sample, remove tubing from the top of the chamber and place a syringe on the top of core and allow the pump to fill the syringe. In order to avoid introduction of oxygen, place top tube into reservoir during

sampling. Sample will be used to measure dissolved oxygen using the mini-Winkler method (see Chapter 5). Another option is to sample and analyze carbon dioxide and methane to get an estimate of anaerobic metabolism (Baker *et al.* 2000, Crenshaw *et al.* 2002).

  f. Using a linear regression approach, calculate the rate of dissolved oxygen concentration change over time.
  g. Save contents of chambers for analyses of AFDM.
3. Estimate surface (benthic) and subsurface (hyporheic) components of system metabolism at upwelling and downwelling locations, using data collected from chamber incubations.
  a. Titrate dissolved oxygen, if required.
  b. Calculate surface net primary production (P_N) in mg O_2 m^{-2} h^{-1}:

$$P_N = \Delta DO \cdot \frac{V}{A \cdot t} \tag{33.2}$$

where ΔDO = change in oxygen during light incubation (mg/L), V = volume of water in chamber (L), A = area of sediment sampled (or of cobbles used) (m^2 = cm^2/10,000), and t = incubation time (hr).

  c. Calculate hyporheic respiration (R) in mg O_2 m^{-2} h^{-1} or in mg g sediment^{-1} h^{-1}:

$$R = \Delta DO \cdot \frac{V}{w \cdot t} \tag{33.3}$$

$$R = \Delta DO \cdot \frac{V}{A \cdot t} \tag{33.4}$$

where w = dry mass of sediment (g). Expressing respiration per unit sediment mass will allow comparison with published values.

4. Estimate whole-stream hyporheic respiration for the reach as the difference between the open-channel measure of ecosystem respiration and the chamber-derived measurements of benthic respiration.
  a. Calculate an integrated estimate of benthic respiration for the reach. The measurements obtained for sediments from upwelling and downwelling locations could be averaged. Alternatively, an average may be calculated whereby the values from each measurement are weighted by the proportion of area mapped as upwelling or downwelling within the study reach. Of course, there may be considerable area that is not strongly upwelling or downwelling. In this case, depending on the variation in metabolic activity observed, an additional measure of benthic respiration representative of neutral exchange zones may need to be obtained.
  b. Calculate the difference between the ecosystem respiration value measured via the open-channel technique and the integrated estimate of benthic respiration

obtained via the chamber method. Calculate the percentage of the total respiration represented by the hyporheic zone estimate. This is an estimate of the hyporheic zone's contribution to whole-system respiration in the study reach.

D. Data Analysis: Basic and Advanced Methods

1. Compare upwelling and downwelling zones (surface stream) in terms of (a) vertical hydraulic gradient; (b) channel features; (c) primary producer abundance, condition, and types; (d) benthic invertebrate abundance and types; (e) temperature and chemical parameters; and (f) surface (benthic) metabolism. Some of these differences may be examined graphically (e.g., with bar graphs) and, if replicate sites were sampled, tested for significance using t-tests (Sokal and Rohlf 1995).
2. Compare upwelling and downwelling zones (hyporheic zone) in terms of (a) hyporheic invertebrate abundance and types; (b) subsurface temperature and chemical parameters; and (c) hyporheic respiration, again using graphical and statistical methods as in step 1.
3. Compare the whole-stream metabolism estimate for the study reach (obtained via the open-channel method) to those estimates derived from chamber incubations of benthic sediments from upwelling and downwelling locations.
4. Compare estimates of hyporheic zone respiration based on the first (open-channel– benthic chamber) and second (hyporheic chambers only) approaches.
5. For parameters relevant to and measured at all sampling points (temperature, chemical parameters, and dissolved oxygen), a two-way analysis of variance (Sokal and Rohlf 1995) with subsystem (surface, hyporheic) and hydrology (upwelling, downwelling) as main factors may be used to detect significant differences. It may be most effective to compare upwelling and downwelling zones in terms of the *difference* between surface and subsurface environments.

IV. QUESTIONS

1. What channel features are related to location of upwelling and downwelling zones?
2. Is there evidence that exchange influences the distribution of periphyton, macrophytes, riparian vegetation, invertebrates, or fish nests in the surface stream or channel?
3. What factors might explain difference in water chemistry or metabolism between upwelling and downwelling zones in the surface stream?
4. To what factors or processes would you attribute differences in community structure or abundance of hyporheic invertebrates between upwelling and downwelling sites?
5. How do hyporheic water temperature and chemistry vary between upwelling and downwelling sites? What might cause these differences (if any)?
6. Does hyporheic respiration at downwelling sites differ from that at upwelling sites? What might cause these differences (if any)?

7. If you found a large difference in some physical or chemical parameters between surface and subsurface waters at upwelling zones, but a small difference in the same parameters at downwelling zones, how would you explain this? How would you explain the reverse?

8. Based on your estimates, how much does hyporheic respiration contribute to whole stream ecosystem metabolism in your study reach?

9. How would total ecosystem metabolism rates differ in a system with only weak hyporheic exchange versus one with strong exchange? Other than the magnitude of exchange, what factors might influence the contribution of the hyporheic zone to ecosystem metabolism?

V. MATERIALS AND SUPPLIES

Mapping

> Clipboard with gridded paper
> Flagging and markers (for labeling flagging)
> Items for flotation (for measuring discharge)
> Meter stick(s)
> Meter tape(s)
> Stakes
> Stopwatch or wristwatch with timer

Hydrology Measurements

> 40-cm minipiezometers (5–10)
> 48-cm well
> 50-cm T-bar
> Biodegradable, nontoxic dye (e.g., fluorescein) in syringe
> Hand pump
> Hydraulic potentiomanometer (Figure 33.2B)
> Small sledge hammer
> Stopwatch

Mapping and Sampling Biota

> 62 μm-mesh nets or sieves
> 70% ethanol and squirt bottle
> Corers (5–10 cm diameter; one or more each for algae and invertebrates)
> Cooler with ice (for unpreserved samples)
> Sample bags (for algae)
> Sample vials or jars (for invertebrates)
> Small bucket or plastic pitcher
> Small ruler for measuring plant/algal heights

Measuring Temperature and Dissolved Oxygen

> 48-cm (or longer) wells (enough to leave in place during sampling)
> 50-cm (or longer) T-bar
> Dissolved oxygen bottles (60-mL size or smaller for low hydraulic conductivity sites)

Dissolved oxygen reagents (see Chapter 5)
Dissolved oxygen trap with hand-operated vacuum pump
Non-iced cooler or box for oxygen samples (to keep dark)
Small sledge hammer
Thermometers (see Chapter 5)
Alternative: dissolved oxygen meter, probe, and collection vessel
Alternative: microwinkler collection vessels (10-mL syringes)

Sampling Nutrient Chemistry and Hyporheic Invertebrates

4-L heavy-walled polyethylene bottle (or other heavy plastic; thin-walled materials will C62 μm-mesh net or sieve)
Cooler with ice
Diaphragm pump mounted on board or bucket (to keep out of water; if mounted on bucket, may invert bucket to sit while pumping)
Hoses to pump and well
Lid for polyethylene bottle fitted with two hose connectors (for pump and well hoses)
Plastic bags or jars (for invertebrates)
Squirt bottle (field wash bottle)
Water chemistry bottles
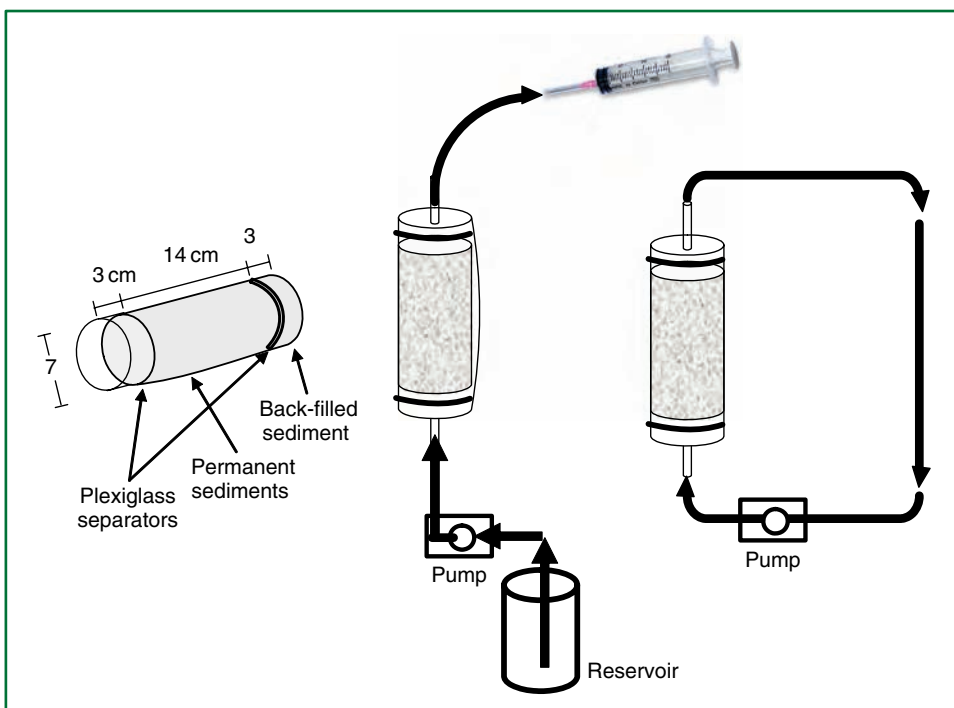
FIGURE 33.3 A schematic of a recirculating chamber design used to measure respiration rates in hyporheic sediments. Panel (A) represents recirculating core design (B) represents sampling mode and (C) represents recirculating mode, arrows depict flow of water. (Adapted from Baker *et al.* 2000.)

Measuring Open Channel Metabolism

> Dissolved oxygen measurement equipment
> Well sampling equipment
> Meter stick
> Solute injection equipment
> Peristaltic pump, tubing, and battery
> Carboy with conservative tracer solute
> Propane or other volatile gas, tubing, and air-stone
> Gas sampling equipment

Measuring Surface Metabolism and Hyporheic Respiration

> Black plastic
> Buckets or carboys for reservoir of "initial" water
> Buckets to transfer materials to be incubated
> Chambers (20)
> Dissolved oxygen measurement equipment
> Well sampling equipment

Hyporheic recirculating chambers

> Buckets or core samplers to obtain sediment
> Chambers and pump (see Figure 33.3) $n = 20$
> Dissolved oxygen measurement equipment
> Bucket for water for cores
> Well sampling equipment
> Black plastic

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Section F

Ecosystem Quality

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Ecological Assessments with Benthic Algae

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I. INTRODUCTION

Integrated ecological assessments provide the information needed to answer a series of questions for managing ecosystems (Stevenson *et al.* 2004a). What are the conditions of valued attributes in the ecosystem? Are they good enough or are they so degraded that we should plan and implement restoration efforts? If conditions are degraded badly, what pollutants need to be reduced and what human activities are generating the pollutants? Thus, ecological assessments can involve much more than measuring biological conditions. They can also characterize and diagnose pollutants and human activities that may be causing problems.

Excessive algal growth and changes in species composition have long been used to assess ecological conditions in stream. Early assessments inferring water quality based on occurrence of pollution sensitive or tolerant species were common in Europe (Kolkwitz and Marsson 1908). As the second half of the twentieth century started, Patrick *et al.* (1954) introduced new concepts with the use of species richness and evenness of assemblages as indicators of biological condition. Diatoms and other algae have been used as indicators of ecological conditions in streams, lakes, wetlands, and coastal zones around the world (Stevenson and Smol 2002).

Algae are important in ecological assessments because they are valued ecological attributes, sources of problems, and good indicators. Algae are an important base of food webs in most aquatic ecosystems (Minshall 1978). Excessive accrual of algal biomass can cause problems by depleting dissolve oxygen supplies, altering habitat structure for aquatic invertebrates and fish, generating taste and odor problems in drinking water

supplies, and producing toxic substances with effects that have not been thoroughly investigated (Palmer 1962, Holomuzki and Short 1988, Carmichael 1997). Algal assessments can more precisely characterize some environmental conditions (e.g., nutrient concentrations, Stevenson 2001) in aquatic ecosystems than one-time sampling because (1) species are particularly sensitive to some environmental conditions; (2) different species have different sensitivities to contaminants; and (3) the development of species composition of algal assemblages takes long enough that they integrate the temporal variability in physical and chemical conditions that vary diurnally and with weather-related events (e.g., runoff and floods).

Many characteristics of algal assemblages are used in ecological assessments (see review in Stevenson and Smol 2002). Biomass of algae can be estimated by direct visual assessments (e.g., Secchi disk and rapid periphyton surveys) and by sampling algae from known areas of substratum or volumes of water with subsequent assays of ash-free dry mass (AFDM), chlorophyll *a* (chl *a*), cell densities, and cell volumes (Humphrey and Stevenson 1992, Stevenson *et al.* accepted). Nutrient content, nutrient biomass ratios, and pigment ratios have been used to predict nutrient limitation and health of algal assemblages in streams (Humphrey and Stevenson 1992, Peterson and Stevenson 1992). Species composition of algae in samples and species' environmental sensitivities, optima, and tolerances have been used in weighted average indicators of biotic condition and contaminants (Potapova *et al.* 2004). Historically, diatoms have been used more in assessments than cyanobacteria, green algae, and other types of algae because they are relatively easy to identify to species level and most algal species in streams are diatoms. However, recent work has shown that using all types of algae in assessments may improve the range of conditions that can be detected (Leland and Porter 2000).

As implied above, algae have been used to assess dependent variables, such as the biotic condition of ecological systems and ecosystem services, as well as infer the contaminants that are causing problems in streams (the independent variables). Multivariate ordination approaches have been used to relate changes in algal species composition to physical and chemical factors altered by humans (Pan *et al.* 1996, 2000). Multimetric approaches have also been used in which scores of different indicators are integrated to reflect the many kinds of changes that can occur in algal assemblages in response to different types of human disturbance (Hill *et al.* 2000, Fore 2002, Wang *et al.* 2005). Many algal metrics are, in one form or another, weighted average metrics and are calculated with a slightly modified version of a formula originally used by Zelinka and Marvin (1961):

$$X = \sum_{i=1}^{S} \theta_i p_i / \sum_{i=1}^{S} p_i \qquad (34.1)$$

Here, X is the indicator, θ_i is an indicator value for the ith species, and p_i is the frequency of occurrence of the ith species in the sample and with an indicator value. If taxa do not have indicators values, they are not included in the calculation. To understand this equation, imagine that we characterized the autecologies of species as oligotrophic, mesotrophic, or eutrophic indicating their nutrient requirements. We could assign numeric values for the trophic autecological characterizations to each species of 1, 2, and 3, respectively. If all organisms in a sample from a site were characterized with the same trophic status, then the value of the indicator would equal 1.0, 2.0, or 3.0. If, for example, half of the

organisms in the sample were characterized as oligotrophic (1) and the other half was mesotrophic (2), then the indicator value would equal 1.5. Thus, the indicator value is the average of indicator values of species after weighting for the relative (proportional) abundance of species with different autecological categories.

This chapter will introduce the breadth of techniques used in ecological assessments of stream algae. In addition, it will provide an example of an integrated assessment of ecological condition of streams in which biological and pollution criteria are established, biological condition is assessed, and potential pollutants are diagnosed. Information from integrated assessments is important for managing streams because they help guide protection and restoration strategies depending upon the biological condition and pollutants in streams. More details on these subjects can be found in other readings. An excellent review of ecological assessment of streams was recently edited by Barbour *et al.* (2004). Two chapters in the book review design and implementation of assessments (Stevenson *et al.* 2004a, b), and provide background on how elements of the algal assessment exercise are used in ecological assessments. Details about algal assessments in aquatic habitats and streams specifically can be found in Lowe and Pan (1996), Stevenson and Pan (1999), or Stevenson and Smol (2002).

II. GENERAL DESIGN

A. Ecological Assessment

Ecological assessments can be delineated into three stages (Stevenson *et al.* 2004a). The first is the design stage, in which the objectives, important attributes, and likely pollutants should be clearly defined. In addition, a conceptual model should be developed, which sets logical hypotheses relating cause-effect relationships between valued attributes, pollutants, and human activities. Finally, the study design for the assessment is established based on the objectives, conceptual model, and economic constraints on the study. In the characterization stage of assessment, the observed conditions at the assessed site are compared to criteria or expectations for valued attributes and pollutants to determine whether conditions meet or fail expectations (Stevenson *et al.* 2004b). The third stage of ecological assessment is diagnosing the pollutants that are most likely causing impairment or threaten valued ecosystem attributes.

Many exercises in the different chapters of this book could be modified to accomplish goals of an integrated assessment, as described in this chapter. In particular, Chapters 16, 17, and 32 could be included because they involve quantifying benthic algal species composition, biomass, and nutrient limitation. In addition, other stream methods involving measurement of physical and chemical habitat characteristics (see Chapters 1–6, 9–11) and macroinvertebrate assessment (see Chapters 20, 23, and 25) could be incorporated into a study design accompanying this chapter. Some of these will be suggested during the following discussion of the project plan. At the least, Basic Method 1 (rapid periphyton assessment) and Basic Method 2 (genus-level taxonomic assessment) should be completed because they will provide sufficient information about algal biomass and taxonomic composition to conduct an integrated assessment. Sample collection can easily be integrated with field work associated with other chapters. Advanced Exercises 1-3 are recommended because they provide additional information and experience about algal division biomass and taxonomic composition. The final section is a data analysis exercise designed to demonstrate the calculation of environmental optima and their application to infer environmental conditions.

B. The Project Plan

Algal assessments will be used in this chapter for the following three objectives: (1) characterize expected condition and criteria for assessing algal production and biodiversity; (2) determine whether pollutants are affecting the productivity and algal biodiversity of streams; and (3) diagnose which pollutants are important. Sediments and nutrients are two of the most common pollutants that affect productivity and biodiversity in streams. Sediments reduce productivity in streams by shading, burying, or coating benthic algae. Sediments alter biodiversity by inhibiting species that can not move vertically through sediments. Nutrients stimulate productivity by enabling accrual of higher biomass. Nutrients alter biodiversity by enabling invasion of taxa that require high nutrients concentrations for survival. Therefore, algal biomass and taxonomic composition, nutrients, and sediments will be important to measure.

C. Sampling Plan

Site Selection. Develop criteria for conditions expected in the absence of human disturbance by assessing physical, chemical, and algal conditions in at least three streams in the region with low human disturbance in their watersheds. These reference streams should be selected randomly from the set of all streams that meet qualifications of having low human disturbance in watersheds. The condition of three or more test sites will be evaluated by assessing the same conditions in other streams of the region. Refine the goal of the assessment to either targeted assessment of streams with particular interest or a characterization of regional streams. In targeted assessments of specific streams, streams can be compared to criteria for expected condition to determine whether the targeted stream meets expectations or not. In a regional assessment, streams must be selected randomly from the set of all possible streams in the region. Random sampling enables generalizing results to all streams that were included in the original set of possible sites. The precision of regional assessments will increase with the number of streams sampled. Therefore, increase both the number of reference and test streams sampled corresponding to the number of students in the class.

Field Sampling and Laboratory Assays. Algal biomass should be measured by using rapid periphyton surveys as described in Basic Method 1. Algal biomass can also be measured with chlorophyll *a*, ash-free dry mass, or algal biovolume as described in other chapters (Chapters 16 and 17; see optional exercises 3 and 4 in this chapter). Taxonomic composition of algae should be measured using methods described in Basic Method 2.

   Relative levels of nutrients and sediments can be inferred based on taxonomic composition of the diatom assemblages characterized in Basic Method 2 and ecological preferences and tolerances provided for some genera. Nutrients can be measured using techniques described in Wetzel and Likens (1991) and in Chapters 9–11. Sediments can be measured using techniques described in Chapter 7 or by the difference between dry mass and ash-free dry mass of benthic algal samples (Chapter 17).

D. Data Analysis Plan

Objective A. Expected condition of physical, chemical, and biological characteristics of ecosystems can be determined many ways (Stevenson *et al.* 2004b). We could expect
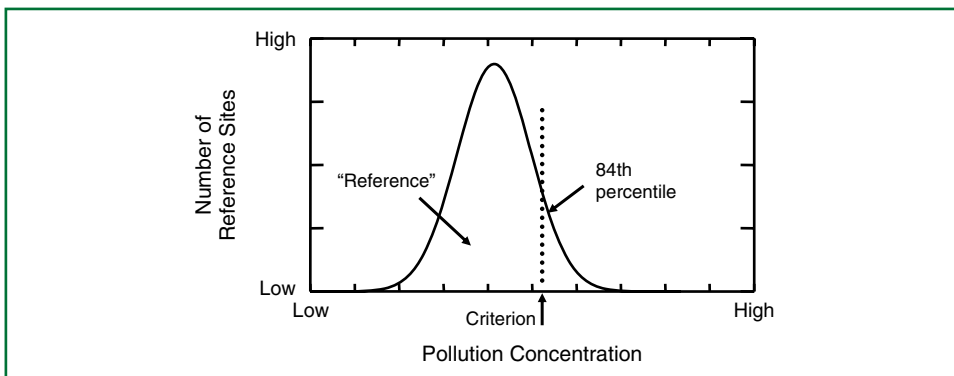
FIGURE 34.1 Criterion for a pollutant based on the 84th percentile of conditions at a reference site. The number of sites with specific concentrations of that pollutant is plotted as a function of the concentration of that pollutant. Sites with pollution concentrations above the criterion would be characterized as impaired for that pollutant. Sites with pollution concentrations below the criterion would be characterized as meeting expectations.

conditions to be as similar to natural as possible, such as the "physical, chemical, and biological integrity" prescribed in the *U.S. Clean Water Act*. Alternatively, we could expect high productivity and many big fish. Thus reaching expectations for different uses of streams may require compromises and different selection criteria for reference sites. For this set of exercises, expected condition will be based on central tendency and variation in conditions at reference sites—that is, those sites with best attainable conditions in the region. Often, either the 25th and 75th percentiles of conditions at reference sites are used as criteria for expected condition (Figure 34.1). For ease of calculation, we recommend using the standard deviation of observations, which is roughly equivalent to the 16th and 84th percentiles. These criteria can be calculated for all attributes, but especially for the important project attributes describing algal biomass, taxonomic composition, nutrients, and sediments.

Objective B. Assessment of conditions at test sites will be done by comparing them with criteria developed from reference sites. If observed conditions at test sites fall within the range delineated by criteria, then we lack evidence to conclude that the test site is impaired. If observed conditions do not meet criteria, the test site is considered impaired.

Objective C. Diagnosing the relative importance of stressors, nutrients, and sediments, in this case, can be estimated as the ratio between the observed condition and the criterion. The greater this ratio, the more likely the stressor is a major cause of problems in impaired streams or threatening sustainability in unimpaired streams.

III. SPECIFIC METHODS

A. Basic Method 1: Rapid Periphyton Survey

Circumstances may prevent the collection, proper preservation, or analysis of algal biomass using chlorophyll *a* or ash-free dry mass. In such cases, rapid periphyton surveys

(RPS) can be used to estimate algal biomass. They can also be used to separate sources of benthic primary production into rough functional categories, which cannot be done using standard chlorophyll *a* and AFDM methods. In this exercise, you will evaluate whether moss and algal growth in test sites differs from that in reference sites using RPS to measure moss and algal biomass. The RPS method presented is a modification of the method described by Stevenson and Bahls (1999) and eliminates the requirement of a viewing bucket.

Field Assessment

1. The RPS will require two people: one "sampler" to evaluate algal biomass and the other to record information on the data sheet provided in Table 34.1; copies should be made for each site that will be sampled.
2. Within the stream reach, establish five transects that cross perpendicular to the direction of stream flow. Transects should be spaced relatively evenly and far enough apart to span the full reach. Assessments of reaches should be restricted to riffles or runs if they are available.
3. Along each transect, you will sample 10 evenly spaced points, beginning with the downstream transect. For narrow streams, you may wish to use more transects, each containing fewer sampling points. Reach down and touch the substratum with your index finger. Do this without looking. If possible, pick up the first substratum that your finger touches. If you cannot remove the substratum, try to evaluate algal growth without removing it.
4. For each of these points, evaluate moss cover, macroalgal cover, and microalgal biofilm thickness using the scales in Table 34.2. In addition, determine suitabililily of substrata for algal accrual by putting a check in the Sz column of the table if substrata are >2 cm in diameter in their longest dimension. Shout your estimates of moss cover, macroalgal cover, microalgal biofilm thickness, and substratum suitability to the data recorder. The recorder should repeat the list of scores while entering them on the data sheet. If the information being repeated back to the sampler is incorrect, the numbers should be corrected before moving to the next sampling point. If the sampler is unable to make these evaluations for a given point, the recorder should mark "NA" in the appropriate columns on the data sheet. If substrata are less than 2 cm in diameter in their longest dimension, do not record information about mosses, macroalgae, or microalgae.
5. Calculate RPS metrics for algal biomass using Table 34.3. Determine extent of moss, macroalgae, and microalgae cover of the stream bottom by dividing the number of substrata with greater than 0 moss, macroalgae, or microalgae observed by (50 − the number of NA). Determine the magnitude of moss and algal biomass on substrata by calculating the products of the number of substrata and ranks of cover; summing the products individually for moss, macroalgae, and microalgae; dividing the sums of the products by the number of substrata of suitable size for algal accrual.

B. Basic Method 2: Genus-Level Periphyton Assays and Index of Biotic Condition

In this method, you will obtain periphyton samples in the field and then subsample them for different laboratory assays, including chlorophyll *a*, AFDM, and taxonomic composition. Start by surveying the reach to determine the best habitat for sampling.

TABLE 34.1 Field Data Sheet for Rapid Periphyton Survey and Algal Sample Collection. Trns = transect number; Macro = macroalgae; Micro = microalgae; Sz = check column to indicate substratum >2 cm.

Stream: _____ Date: _____ Sampler: _____ Recorder: _____

Point	Trns	Moss	Macro	Micro	Sz	Point	Trns	Moss	Macro	Micro	Sz
1						26					
2						27					
3						28					
4						29					
5						30					
6						31					
7						32					
8						33					
9						34					
10						35					
11						36					
12						37					
13						38					
14						39					
15						40					
16						41					
17						42					
18						43					
19						44					
20						45					
21						46					
22						47					
23						48					
24						49					
25						50					

Total Algae Sample Volume = _____   Surface Area Sampled = _____
Identification Subsample Volume = _____   Substrata Sampled: rock/wood/plant
Chlorophyll Subsample Volume = _____   (circle)   sand/silt/other
AFDM Subsample Volume = _____

TABLE 34.2 Algae and Moss Cover and Thickness Class Descriptions.

Moss and Macroalgae Cover Classes

Class	0	1	2	3	4
Cover	0%	<5%	5% to 25%	25% to 50%	>50%

Microalgae Thickness Class

Class	0	1	2	3	4	5
Thickness	0 mm	<0.5 mm	0.5 to 1 mm	1 to 5 mm	5 to 20 mm	>20 mm
Characteristics	rough	slimy; visible evidence of biofilm absent	biofilm visible; may require scraping of surface			

TABLE 34.3 Calculations for Extent and Magnitude of Moss and Benthic Algal Cover. Shaded areas do not have appropriate records or calculations.

Rank	Moss		Macroalgae		Microalgae	
	No	Rank × No	No	Rank × No	No	Rank × No
1						
2						
3						
4						
5						
NA						
(No > 0) ÷ (50−NA)						
Sum ÷ (50−NA)						

The habitat should be shallow enough to have sufficient light to support benthic algal growth. You should be able to see algae on the substrata.

Collecting Periphyton Samples

1. Prioritize the habitat selected for sampling using the following three criteria: (a) select rock, wood, or plant (firm) substrata in current velocities greater than 15 cm/s, but not too high, to minimize silt in the sample and effects of current on species composition; (b) if you cannot find firm substrata in fast current, sample them in slow current; (c) if sufficient firm substrata are not in the reach, then sample sediments (ranging from sand, to fine detritus, to silt) in slow-current habitats (often along the stream margins) where sediments have been stable and periphyton accrual is evident or at least likely.

2. Sample periphyton on firm substrata by randomly selecting and removing at least five substrata from the stream and placing them in a pan for processing onshore. Scrape periphyton into another white pan from all areas of the substratum that were exposed to light and not buried in sediments. Scrape thick accumulations with a knife or spoon to remove most of the algae and then brush with a toothbrush to remove more tightly attached individuals. Rinse the sampled surface and sampling tools into the pan with distilled water from a squirt bottle. Cut long filaments of algae into short pieces with scissors. Rinse all algae from the pan into a subsampling bottle marked with volumetric graduations. Fill the subsampling bottle with distilled water to the next volumetric graduation. Record the total volume of the sample on the field data sheet (Table 34.1). Measure the area of sampled surfaces of substrata with a ruler. Record area sampled on the field data sheet.

3. If firm substrata are not available, sample periphyton on sediments using a Petri dish and spatula from at least five representative locations of the targeted habitat in the stream. Insert a dish into the sediment in the stream and slide the spatula under the open end of the dish to capture a short core of sediments. Remove the sediments from the stream, invert the core, and rinse the core from the dish into the 1-L sample bottle. If the sediment is organic and fine, and algae are not expected deep within the sediment, the bottom of the core could be removed to reduce silt from the final sample. If the sediment is sandy or coarser, a swirl-and-pour technique should be employed to remove algae from the coarse inorganic substrata before subsampling the collected periphyton (Stevenson and Stoermer 1981). The swirl-and-pour technique removes algae from coarse substrata by repeatedly adding small amounts of water to the sediment sample, swirling the sample to tumble sediments and thereby scouring algae from the fine substrata. Gently pour the suspended algae from the sample to a white pan. This step should be repeated from 5 to 10 times or until the poured water appears relatively clean. Empty and rinse the 1-L sample bottle and pour the algal suspension from the white pan into the graduated subsampling bottle. Rinse the pan with deionized water into the subsampling bottle. Fill the subsampling bottle with distilled water to the next volumetric graduation. Record the total sample volume on the field data sheet. Record the area sampled on the field data sheet.

Subsampling for Different Assays

Algal identification, cell counting, and AFDM assessments require preservation (but see Chapter 17 for an alternate AFDM method). Pigment analysis requires sample storage in the field on ice, and freezing in the laboratory until assayed.

1. Subsample separately for algal assays that require preserved or unpreserved sample. Subsampling should be done by removing two or more aliquots with a turkey baster or large pipette and placing them in a sample bottle. Record the volume of the subsample in the sample bottle on the field data sheet (Table 34.1).
2. Subsample a relatively large proportion of the total sample for microscopic and AFDM analysis. Preserve these samples with 3–5% buffered formaldehyde or gluteraldehyde, or take a separate subsample for AFDM and process according to Chapter 17.
3. Subsample the algal suspension for chlorophyll *a* and pigment analysis and place this sample on ice in the dark until returned to the lab. Freeze the pigment samples

(or filters with periphyton; see Chapter 17) in the lab if they will not be analyzed immediately. Check for correct labels on the sample bottles before leaving the sample site. Unused sample remaining after subsampling may be discarded.

Identifying Diatom Genera and Counting Cells

Although assessing taxonomic composition of algal assemblages usually calls for identifications to the species level of taxonomy, recent papers have shown the utility of genus-level assays (Hill *et al.* 2001, Wang *et al.* 2005). Genus-level assays may not be as precise as species-level assays and assessment of biological condition, but they do have value and can be conducted with less technical expertise (see Chapter 16). Furthermore, diatom taxa are particularly useful for assessing ecological condition. In this exercise, we assume that genus-level metrics for diatoms will transfer from the regions and stream types in which they were developed to study streams selected for your project.

1. Clean diatoms and mount them in a high resolution mounting medium as recommended in Chapter 16. Using the genus-level key provided in that chapter, identify the diatom genus of each valve and count 300 valves of diatoms in random fields around the coverglass. Record the genera and number of valves observed on Table 34.4.
2. *Option*: keep track of the sample volume put on the coverglass and number of fields counted to determine the numbers of diatoms per area of substratum as detailed in

TABLE 34.4	Bench Sheet Example for Cleaned Diatom Counts.

Stream: _____ Date: _____ Counter: _____

Fields Counted: _____

Genus Name	Valve Count	Cell Count (valves ÷ 2)
Genus 1		
Genus 2		
.		
.		
.		
.		
.		
.		
.		
Genus n		

Total Sample Volume = _____ mL
Volume Cleaned = _____ mL
Volume on Coverglass = _____ mL

Advanced Method 1. Be careful to distinguish whether one or two valves are present in the diatom cell wall (frustule) and whether one or more cells are aggregated together.

Calculating Diatom Metrics

1. Using the diatom benchsheet table (Table 34.4), tally the number of valves observed for each genus of diatom and divide by 2 to get the number of cells of each genus (n_i). Sum the n_i to determine the total number of cells observed (N) in the count. Calculate the proportional relative abundance of each ith genus (p_i) of diatom as $p_i = n_i/N$.

2. Calculate the following metrics to assess biotic condition of diatom assemblages: % *Achnanthes*, % *Cymbella* plus *Encyonema*, % *Navicula*, and % *Nitzschia*. Contrary to suggestions in Stevenson and Bahls (1999), high relative abundances of *Achnanthes* are characteristic of low nutrient reference streams in many regions of the country, particularly *A. minutissima* and *A. biasolletiana* (Wang *et al.* in press, R. J. Stevenson unpublished data). *Cymbella* and *Encyonema* are also common in references streams, while *Navicula* are more common in disturbed streams (Wang *et al.* 2005). *Nitzschia* are pollution tolerant (Hill *et al.* 2001). Record these in the assessment table (Table 34.8).

3. Calculate the following metrics to infer relative pollution conditions: % acidobiontic, % eutraphentic, % motile. According to Hill *et al.* (2001), the average environmental preference of species in the genera *Eunotia*, *Frustulia*, and *Tabellaria* would classify them as acidobiontic, meaning taxa with tolerances for pH < 5.5 (Lowe 1974, van Dam *et al.* 1994). On average, species in the genera *Amphora*, *Cocconeis*, *Diatoma*, *Gyrosigma*, *Meridion*, *Nitzschia*, and *Synedra* are eutraphentic, meaning taxa with requirements for nutrient-enriched waters (van Dam *et al.* 1994). *Navicula*, *Nitzschia*, *Surirella*, *Cymatopleura*, and *Gyrosigma* are relatively common genera that are motile and commonly found in sediments. Relatively rare and planktonic genera with acidobiontic or eutraphentic environmental preferences are not listed above. Record metrics in Table 34.8.

C. Advanced Method 1: Identify and Count All Algae

In this exercise, you will identify all algae, not just diatoms, to a coarse taxonomic level along with their relative cell size. High biovolumes and percent biovolumes of cyanobacteria usually indicate nutrient and organic enrichment by human activities, and a high percentage of diatoms is considered more natural (Hill *et al.* 2000). However, any deviation from reference condition should be considered a decrease in biological condition.

1. Prepare a Palmer Counting Chamber with 0.1 mL of algal suspension from a known proportion of the original sample.

2. Determine the size and division of algae for 300 cells in random fields around the counting chamber. Record the number of cells of green algae, cyanobacteria, diatoms, and other algae by size category in Table 34.5. Do not include diatoms without protoplasm in the frustules in your count of 300 cells, as these are considered dead.

TABLE 34.5 Bench Sheet for All Algae Counts.

Stream: _____ Date: _____ Counter: _____

Fields Counted: _____

Taxonomic Size Class	Biovolume Weight	Cell Count
Diatoms		
Width <5 μm	4	
5 μm ≤ width <12 μm	6	
Width ≥12 μm or >25 μm long	8	
Dead Diatoms	0	
Greens		
Width <5 μm, length ≤5 μm	3	
Width ≥5 μm, length ≤5 μm	5	
Width <50 μm, length >5 μm	4	
Width ≥5 μm, length >5 μm	8	
Cyanobacteria		
Width <3 μm, length ≤5 μm	1	
Width ≥3 μm, length ≤5 μm	3.5	
Width <3 μm, length >5 μm	3	
Width ≥3 μm, length >5 μm	5.5	
Other		

Palmer Chamber Dilution Factor = _____
    (Volume of Deionized Water ÷ Volume of Sample)
Total Algae Sample Volume = _____ mL
Stream Surface Area Sampled = _____ cm^2
Microscope Field-of-View Area = _____ μm^2
Palmer Chamber Depth = _____ μm
Proportion of Palmer Chamber Counted = _____
    (# Fields × Field-of-View Area × Palmer Chamber Depth) ÷100 μL Palmer Volume
Volume of Original Sample Counted = _____ mL
    (Proportion of Palmer Counted × 0.1 mL Palmer Volume) ÷ Dilution Factor
Stream Area Counted = _____ cm^2
    (Original Vol. Counted ÷ Total Algae Sample Volume) × Stream Area Sampled
Cell Density = _____ cells/cm^2
    (Number of Cells Counted ÷ Stream Area Counted)

3. Normally, biovolume of each species is assessed (see Chapter 16). However, when only identifying to division, use the size categories provided to group algae with relatively similar biovolumes.
4. From the data in Table 34.5, calculate some useful algal metrics. Calculate cell densities and biovolumes of all algae, cyanobacteria (blue-green algae), and diatoms using equations at the bottom of Table 34.5. Calculate the percent of biovolume represented by cyanobacteria and diatoms.

D. Advanced Method 2: Species Autecologies and Inferring Environmental Condition

Weighted average models are one of the simplest and most tested techniques for inferring environmental conditions based on species composition and their optimal environmental conditions. This technique has been used to characterize historic conditions in lakes using species composition in sediment records and to infer nutrient concentrations in streams where it can be so variable (Potapova *et al.* 2004, Stevenson and Smol 2002). Developing weighted average models requires two steps: (1) characterizing species environmental optima and (2) testing and calibrating the model. In this exercise, you will first calculate the optimum environmental condition for one species, model calibration information will be provided, and then you will apply the model to infer environmental conditions in a stream based on species composition and their environmental optima.

Calculating an Environmental Optimum for a Species

Species environmental optima are calculated using a calibration dataset and a weighted average model. The calibration dataset is composed of species abundances and environmental characteristics at sites throughout a region. The weighted average model for a species' environmental optimum is:

$$\theta_i = \sum_{j=1}^{N} E_j p_{ij} / \sum_{j=1}^{N} p_{ij} \qquad (34.2)$$

In the model, the environmental optimum of the ith species (θ_i) is the sum of the products of ith species abundance at site j (p_{ij}) and measured environmental conditions at site j (E_j) for all N sites, divided by the sum of the ith species' abundance for all sites.

Using data provided in Table 34.6, calculate the total phosphorus optimum for *Navicula cryptocephala* Kützing.

1. Determine the products of species abundance (p_{ij}) and measured environmental conditions (E_j) for all sites.
2. Sum the species abundances and products of species abundances and environmental conditions, independently.
3. For this example, determine the total phosphorus optimum for *N. cryptocephala* by dividing the sum of the products of species relative abundances and total phosphorus concentrations by the sum of the species relative abundances.

TABLE 34.6	Proportional Relative Abundances (PRA) of *Navicula cryptocephala* and Total Phosphorus Concentrations at 17 Stream Sites.			
Taxon	Site #	PRA (p_i)	TP (E_i)	PRA × TP ($p_i \times E_i$)
Navicula cryptocephala	1	0.05	55	
Navicula cryptocephala	2	0.15	25	
Navicula cryptocephala	3	0.10	10	
Navicula cryptocephala	4	0.10	45	
Navicula cryptocephala	6	0.20	35	
Navicula cryptocephala	8	0.10	5	
Navicula cryptocephala	9	0.10	20	
Navicula cryptocephala	10	0.40	30	
Navicula cryptocephala	12	0.05	15	
Navicula cryptocephala	13	0.15	50	
Navicula cryptocephala	14	0.05	5	
Navicula cryptocephala	15	0.10	60	
Navicula cryptocephala	16	0.35	30	
Navicula cryptocephala	17	0.10	40	
Sums				
Optimum				

Calculating Inferred Total Phosphorus Concentration for a Stream

Using data in Table 34.7 and Equation 34.1, calculate the inferred total phosphorus concentration for a stream.

1. Record relative abundances of species for which the total phosphorus optimum is known (p_{ij}).
2. Calculate the products of the species relative abundances and their total phosphorus optima.
3. Sum the proportional relative abundances of species for which total phosphorus is known and the products of species relative abundances and total phosphorus optima.
4. Calculate inferred total phosphorus concentrations for the stream as by dividing the sum of the products of species relative abundances and total phosphorus by the sum of the species relative abundances for which total phosphorus optima are known.

TABLE 34.7 Proportional Relative Abundances (PRA) and Total Phosphorus Optima (TP_{Opt}) of Diatom Species in a Benthic Algal Sample from a Stream.

	PRA	PRA with TP_{Opt} (p_i)	TP_{Opt}	PRA × TP_{Opt} (θ_i)
Navicula reichardtiana	0.2		34	
Nitzchia frustulum	0.2		71	
Amphora perpusilla	0.1		67	
Hippodonta capitata	0.1		44	
Navicula cryptocephala	0.05		31	
Gyrosigma acuminatum	0.1		59	
Gomphonema angustatum	0.05		66	
Gomphonema parvulum	0.05		50	
Achnanthidium minutissimum	0.05		33	
Staurosirella lapponica	0.1			
Sums				
Inferred TP				

E. Advanced Method 3: Biomass Assays

1. Use the subsamples saved during sampling for AFDM and chlorophyll *a* analysis described in Basic Method 2. Follow instructions in Chapter 17 to determine AFDM and chlorophyll *a* in the subsamples.
2. Calculate the proportions of total samples represented by AFDM and chlorophyll *a* subsamples by dividing the volume used in each analysis by the total sample volume recorded on the field data sheets.
3. Calculate area of substrate from which the subsamples were collected by multiplying these proportions by the area sampled.
4. Calculate AFDM and chlorophyll *a* per unit area by dividing AFDM and chlorophyll *a* in samples by the area of substrate from which the subsamples were collected.

F. Analysis and Interpretation of Data

Determining Expected Conditions

1. List all the attributes of algal biomass and taxonomic condition, algal inferred stressor conditions, and measured nutrient concentrations and sediments in the assessment table (Table 34.8). Include division biovolumes and percent biovolumes if determined. Predict whether human activities would increase or decrease each attribute and mark it in the assessment table.
2. Calculate and record the mean and standard deviation of all attributes at reference sites. Calculate and record the 16th and 84th percentiles of each attribute by, respectively, subtracting and adding the standard deviation from the mean.

TABLE 34.8 Assessment Calculation Table. MT refers to metric type where BC is a metric of biological condition and IPC is a metric that infers pollution condition.

Metric	MT	Reference Mean	Lower Criterion (e.g., 16%)	Upper Criterion (e.g., 84%)	Site Value	Site Value ÷ Ref. Mean	Meets Criterion? (yes/no)
% *Achnanthidium*	BC						
% *Cymbella + Encyonema*	BC						
% *Navicula*	BC						
% *Nitzschia*	IPC						
% Acidobiontic Genera	IPC						
% Eutraphentic Genera	IPC						
% Motile Genera	IPC						
Moss Cover Extent	BC						
Moss Cover Magnitude	BC						
Macroalgae Cover Extent	BC						
Macroalgae Cover Magnitude	BC						
Microalgae Cover Extent	BC						
Microalgae Cover Magnitude	BC						
Chlorophyll *a*	BC						
AFDM	BC						
Cyanobacteria Density	BC						
% Cyanobacteria	BC						
Cyanobacteria Biovolume	BC						
% Biovolume Cyanobacteria	BC						
Green Algae Density	BC						
% Green Algae	BC						
Green Algae Biovolume	BC						
% Biovolume Green Algae	BC						
Diatom Density	BC						
% Diatoms	BC						
Diatom Biovolume	BC						
% Biovolume Diatoms	BC						

Assessing Stream Conditions for Targeted Streams

1. Compare values of all metrics of biological condition (algal biomass and taxonomic composition) to respective criteria for reference conditions in each targeted stream.
2. Determine the number of metrics for biological condition and pollutants that meet criteria for each targeted stream.

Assessing Stream Conditions for the Region

1. Compare values of all metrics of biological condition (algal biomass and taxonomic composition) to respective criteria for reference conditions.
2. Determine the proportion of test streams in the region that were impaired for each biological condition. Since these test streams were randomly selected from the set of all regional streams (although perhaps a limited size and landscape type), we can assume that this proportion is a reasonable estimate of the proportion of all regional streams that are impaired for these conditions.

Diagnosing Likely Causes of Impairment

1. Calculate ratios of all biologically inferred and measured pollutants (acidification, nutrient enrichment, and sediment) in each test stream with criteria for reference conditions.
2. For each impaired stream, determine whether pollution ratios are greater or less than 1 and therefore, which pollutants are likely impairing stream conditions. For targeted streams, this information can be used to restore streams. With randomly selected streams, the proportion of regional streams impaired and threatened by different stressors can be determined.

IV. QUESTIONS

1. Researchers have long suggested that species composition should be more sensitive to environmental change than aggregate community properties such as primary production. Why might this be true? Do your data support these assertions?
2. Did you have any difficulty finding good reference sites to assess your test sites? In regions where this is a problem, how might water quality criteria be developed?
3. The nature of many environmental problems prevents traditional experimental approaches. Thus, environmental assessments often take a survey approach similar to those used in epidemiology. How do survey approaches differ from more traditional scientific experiments? What are the pros and cons of each approach? How might inferences drawn from surveys be strengthened? Can hypotheses be tested using both approaches?
4. For your impaired streams, which stressors are most important? For unimpaired streams, which stressors most threaten impairment? How might these affect other organisms using these streams? How might these affect various uses of these streams by humans?
5. Are there alternative explanations for the patterns you observed? How would you design a study to determine which explanations are most probable?
6. What management actions would you recommend to improve or maintain the water quality of these streams?
7. Estimates of standard deviation are influenced by sample size. How might this influence water quality criteria if percentiles are estimated as above? How would an increased number of test sites influence the probability of declaring the region impaired? How might this influence monitoring decisions?

V. MATERIALS AND SUPPLIES

Field

Spoon or knife
Toothbrush
Scissors
2 pans (white plastic dish tubs works well)
Squirt bottle filled with deionized water
1-L sample bottle with marked volumetric graduations
Metric ruler
Petri dish (one-half)
Kitchen spatula (without openings)
Graduated sample bottles (plastic screw-cap centrifuge tubes work well)
Turkey baster
Formaldehyde or gluteraldehyde
Pipette for dispensing known volume of preservative
Labels
Permanent markers
Data sheets
Pencils

Laboratory

Light microscope
Bench sheets
Slides
Coverglass
Mounting medium
Palmer counting chamber
10–100 µL adjustable pipette
100–1000 µL adjustable pipette
Optional supplies and equipment for chlorophyll *a* and AFDM (listed in Chapter 17)

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Macroinvertebrates as Biotic Indicators of Environmental Quality

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I. INTRODUCTION

The use of aquatic organisms to assess water quality is a century-old approach (Kolkwitz and Marsson 1909, Cairns and Pratt 1993), but monitoring programs in North America relied mainly on chemical and physical monitoring until the 1970s. One problem in relying solely on chemical and physical measurements to evaluate water quality is that they provide data that primarily reflect conditions that exist when the sample is taken. In essence, a physico-chemical approach provides a "snapshot" of water-quality conditions. In contrast, biological monitoring provides a "moving picture" of past and present conditions, and hence, a more spatially and temporally integrated measure of ecosystem health.

Of all the freshwater organisms that have been considered for use in biological monitoring, benthic macroinvertebrates (mainly consisting of aquatic insects, mites, molluscs, crustaceans, and annelids) are most often recommended (Hellawell 1986, Bonada *et al.*

TABLE 35.1	Advantages and Difficulties to Consider in Using Benthic Macroinvertebrates for Biological Monitoring (summarized from Rosenberg and Resh 1993, who also discuss how to overcome the difficulties mentioned.)

Advantages	Difficulties to Consider
1. Being ubiquitous, they are affected by perturbations in all types of waters and habitats	1. Quantitative sampling requires large numbers of samples, which can be costly
2. Large numbers of species offer a spectrum of responses to perturbations	2. Factors other than water quality can affect distribution and abundance of organisms
3. The sedentary nature of many species allows spatial analysis of disturbance effects	3. Seasonal variation may complicate interpretations or comparisons
4. Their long life cycles allow effects of regular or intermittent perturbations, variable concentrations, etc., to be examined temporally	4. Propensity of some macroinvertebrates to drift may offset the advantage gained by the sedentary nature of many species
5. Qualitative sampling and analysis are well developed, and can be done using simple, inexpensive equipment	5. Perhaps too many methods for analysis available
6. Taxonomy of many groups is well known and identification keys are available	6. Certain groups are not well known taxonomically
7. Many methods of data analysis have been developed for macroinvertebrate assemblages	7. Benthic macroinvertebrates may not be sensitive to some perturbations, such as human pathogens and trace amounts of some pollutants
8. Responses of many common species to different types of pollution have been established	8. Poorly established relationships between specific stressors and most commonly used metrics
9. Macroinvertebrates are well suited to experimental studies of perturbation	
10. Biochemical and physiological measures of the response of individual organisms to perturbations are being developed	

2006, Carter *et al.* 2006). For example, 49 of the 50 states in the United States use macroinvertebrates in water-quality monitoring (and the 50th is developing a program), whereas only about two-thirds of the programs use fish, and only one-third use algae (USEPA 2002). Even though there are many advantages to using macroinvertebrates in water-quality monitoring, as with all methods of environmental assessment, the disadvantages must also be considered (see Table 35.1).

Macroinvertebrates have been used to evaluate the effects of anthropogenic stressors at all levels of biological organization, from the molecular to the ecosystem (Rosenberg and Resh 1993). At the molecular level, the effects of pesticides have been examined by measuring depressions in acetylcholinesterase levels (Buchwalter *et al.* 2004). Likewise, changes in levels of mixed-function oxidases, metallothioneins, and the extent of DNA damage have been shown to be useful in identifying the effects of a variety of stressors (Belden and Lydy 2000, Cain *et al.* 2000). Collectively, these biochemical changes are referred to as biomarkers (Johnson *et al.* 1993).

At the organism level, the changes in growth and reproduction and rates of morphological deformities have been evaluated as responses to increased pollution (Martinez *et al.* 2002). Likewise, various physiological responses, such as changes in respiration,

metabolism, and bioenergetics have been examined in terms of their response to specific pollutants (Buchwalter and Luoma 2005). Many of these organism-based processes have been evaluated in the field but even more have been evaluated in a laboratory setting, often by performing bioassays.

Most commonly, the population and community (= assemblage) levels are evaluated when the effects of pollution are examined in nature. For example, the abundance of populations and the abundance, richness, and evenness of macroinvertebrate assemblages have been routinely examined in water-quality studies for decades. In addition to these structural characteristics, many functional approaches that evaluate life-history characteristics (species traits) are also used in biomonitoring (Gayraud *et al.* 2003, Statzner *et al.* 2005). A taxon's functional feeding group is by far the most widely used species trait evaluated in water-quality studies (see Chapter 25). However, many other species traits can also be used in impact assessment and many are now being tested (Bonada *et al.* 2006). Regardless of whether the measures (metrics) evaluated are structural or functional attributes, assessment proceeds by comparing these values between unimpaired (= reference) sites and putatively impacted (= test) sites. Although bioassessments based on metrics dominate United States programs, many sophisticated multivariate statistical procedures are used to evaluate stream impairment using macroinvertebrate assemblages as well.

The simplicity and low cost of macroinvertebrate collecting and the ease with which water-quality evaluations can be made has lead to considerable development of volunteer monitoring programs in the United States (Ely 2005). Conservation groups such as the Isaac Walton League of America (IWLA) popularized simplified field assessments for use by concerned citizens with the Save Our Streams (SOS) program (Firehock and West 1995). Early USEPA Rapid Bioassessment Protocols (Plafkin *et al.* 1989) also described a cursory, or "RBP I," approach that was generally accepted as suitable for nonprofessionals given that it was based on the IWLA SOS protocol (Firehock and West 1995). The quality of data obtained by volunteers using good equipment (e.g., microscopes, undamaged nets) and adhering to accepted protocols can be very similar to data obtain by professionals when the same techniques are followed (Fore *et al.* 2001). However, the level of training received by volunteers has a significant effect on the quality of laboratory processing and identification. The involvement of professionals and the constancy of personnel in volunteer programs contribute positively to data quality (Ely 2005). Because the taxonomic resolution achieved by volunteer monitoring groups is often not as detailed as professional assessments, the number and types of indices and analyses that can be used for a stream assessment are somewhat limited. However, detailed taxonomy is not necessary for deriving many commonly used metrics.

It is important to recognize that changes in the use of benthic macroinvertebrates in biomonitoring will continue to occur. For example, Bonada *et al.* (2006) evaluated a range of approaches in terms of how they met preestablished criteria of an ideal biomonitoring tool based on their underlying rationale, implementation, and performance. They found that many newer applications performed far better than the oldest, widely used *Saprobian* approach (Niemi and McDonald 2004). Furthermore, debates today on which organisms should be used for aquatic bioassessments (such as fish, macroinvertebrates, or diatoms), taxonomic levels needed (family, genus, or species), and which analytical techniques (summary statistics, univariate approaches, or multivariate approaches) seem far from being resolved. However, large-scale state and federal programs desiring increased comparability may ultimately resolve many of these issues.

Benthic macroinvertebrates represent an integral part of lotic systems by processing organic matter and providing energy to higher trophic levels; therefore, an understanding

of the effects of anthropogenic, as well as natural stressors, on their distribution and abundance is critical for comprehensive impact assessment of streams and rivers. In this chapter we describe the fundamental processes and considerations necessary for using macroinvertebrate assemblages for environmental assessment. We provide two procedures: a basic method that requires effort similar to that used in a student assignment or a volunteer-based, site-specific project; and an advanced procedure that involves effort similar to that used in a graduate student or larger-scale project. An evaluation of stream habitat is also presented because of the importance of habitat in the distribution of macroinvertebrates and because anthropogenic effects on habitat are often the impact of concern.

II. GENERAL DESIGN

The basic principal behind assessing impairment by evaluating the structure and function of macroinvertebrate assemblages is the comparison of putatively (or presumed) impaired sites to unimpaired sites (see also Chapter 36). Unimpaired sites are known as control or reference sites and putatively impaired sites are known as test sites. The phrase "control site" is rarely used today however, and has been replaced by the concepts of reference site or reference condition (see following). Also, it is generally accepted that pristine reference conditions are rarely available in a study area, so comparisons are normally made between putatively impaired sites and least impaired sites.

A fundamental consideration before making comparisons between reference and test sites of attributes derived from the analysis of macroinvertebrate assemblages is that both sets of sites have a similar biological potential in the absence of impact. Therefore, studies are most often restricted to areas that have similar gross physiography. Ecoregions, subecoregions, type of land cover, stream size, and elevation are just a few of the criteria to consider when restricting the range of physical variables that could confound comparisons of macroinvertebrate assemblages among sites in impact assessment. The importance of these variables to the design of a bioassessment is often a function of the scale of the question being addressed.

Spatial and temporal considerations are critical in all assessment designs. In fact, geographic scale often dictates whether a study will follow a point-source or regional assessment design. Small scale, point-source studies frequently use one of many BACI-type (Before-After-Control-Impact) designs (Stewart-Oaten *et al.* 1986). In the simplest case with these designs, comparisons are made before and after an impact occurs at both control (reference) and impacted sites. The comparisons are based on analysis of variance (ANOVA) approaches that assess impact by appropriately partitioning variability. The Canadian Environmental Effects Monitoring (EEM) program website (http://www.ec.gc.ca/eem/english/default.cfm) provides documents that include extensive design criteria for point-source studies. A very readable account of basic experimental design in ecology is presented by Underwood (1997); a more thorough treatment of the many complicating aspects associated with proper impact study design in streams is presented by Downes *et al.* (2002).

Large-scale regional assessments are the foundation of most state and national bioassessment programs. Although, the variety of designs used for these assessments is extensive, the basic principles are similar. In most studies, putatively impaired sites are compared to a reference condition. The use of reference conditions rather than a specific site has become increasing popular (Reynoldson *et al.* 1997, Bailey *et al.* 2004) because this method accounts for the variability among reference sites that are an inherent aspect of all large scale

studies. When reference conditions are unknown or unknowable, gradient-type assessments are often performed and reference conditions can be predicted (Carter and Fend 2005).

Analytical Approaches

The process of analyzing macroinvertebrate assemblage data for bioassessments is often divided into two approaches: multimetrics and multivariate. A principal distinction between the two approaches lies in how variables are defined. Both use the same raw species × sample data matrix; however, in the multimetric approach, the variables (metrics) analyzed are derived by estimating certain summary characteristics from the species × sample data on a per sample basis. These characteristics include the richness or percentage composition of certain taxonomic or feeding groups, measures of species diversity and evenness, and biotic indices based on tolerance scores. Once metrics are estimated, the value of each metric (or a combined multimetric) is compared among samples. Conversely, in the multivariate approach metrics are not estimated as they are in the multimetric approach. Rather, samples are compared by their position in species-space by using the presence (or a measure of abundance) of each taxon in each sample as input to a classification and/or ordination procedure. A wide range of differences exists among programs in the application of these two data analysis approaches; nevertheless, the data necessary for performing bioassessments based on both multimetric and multivariate methods are practically identical (Figure 35.1).

A. Multimetric Approach

The multimetric approach is based on the premise that certain measures of the benthic assemblage can be used to indicate its ecological condition and, by extension, the condition of the stream ecosystem. Many well established metrics have been used in stream assessments. Although an underlying assumption has been that most metrics are firmly based on accepted ecological theory, painfully little testing of this premise has been done.

Most contemporary survey approaches rely on multiple measures of community structure and function. These metrics can be grouped into several categories, such as (1) taxa richness (e.g., family-level, generic-level, species-level) of either the entire benthic assemblage or of specific components that are viewed to be tolerant (e.g., Chironomidae) or intolerant (Ephemeroptera, Plecoptera, and Trichoptera – so-called EPT taxa) to pollution; (2) enumerations (e.g., number of all macroinvertebrates collected) or proportions of selected orders such as the EPT; (3) community diversity indices, which generally reflect dominance (e.g., Shannon's index); (4) functional feeding group ratios (e.g., percentage of the "shredder" functional group; see also Chapter 25); and (5) biotic indices.

An expected change in species richness accompanying impairment is based on the premise that a loss of species occurs with increased impairment. A change in the number or proportion of individuals within a certain taxon is based on the notion that, with some types of pollution, more intolerant individuals may be lost (e.g., EPT), while the numbers of tolerant individuals may rise (e.g., certain species of Chironomidae). Diversity indices summarize richness and evenness (the distribution of number of individuals among the number of species present), and sometimes total density of assemblages into a single number. The functional feeding group concept assumes that organisms that have evolved to use a certain food source (e.g., through the shape of their mouthparts) should be present when those resources (e.g., packs of leaves, algae, or suspended materials) are present. When stressors alter these resources, functional feeding groups are hypothesized
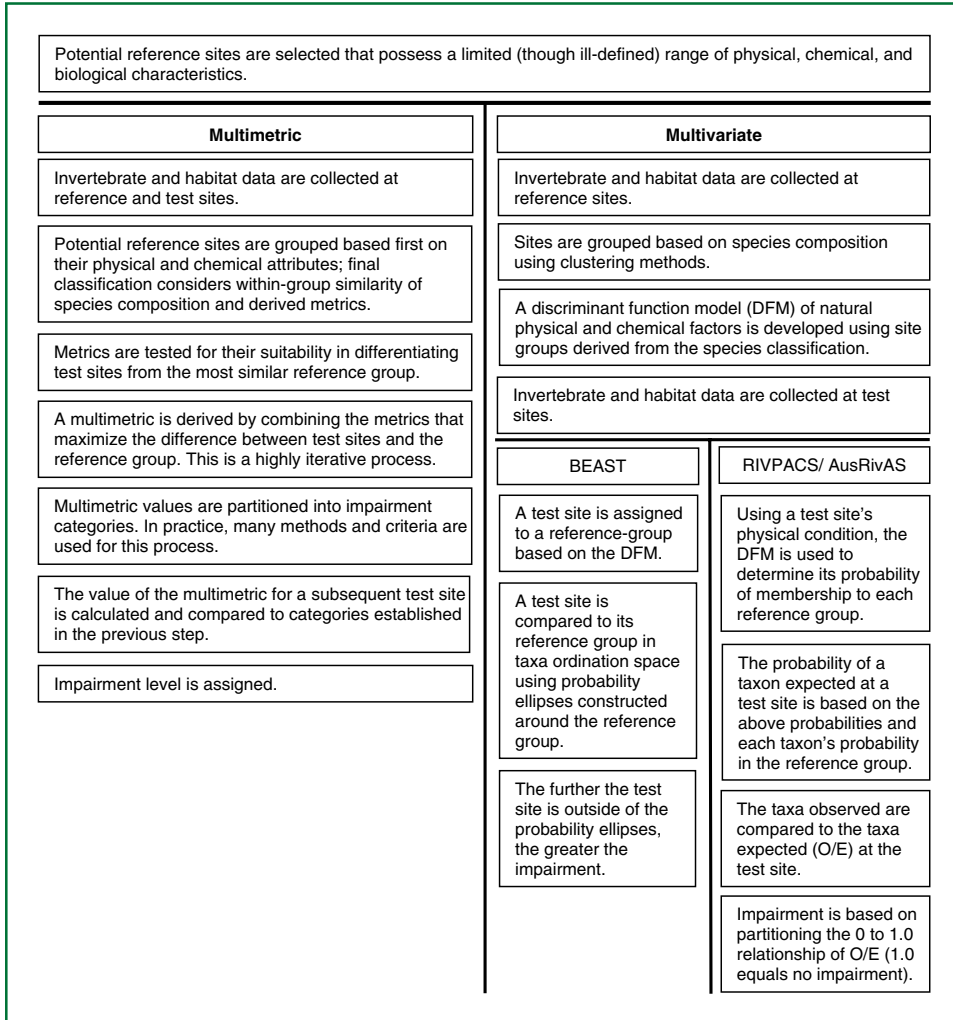
FIGURE 35.1 Comparison of the steps in multimetric and multivariate bioassessments BEAST, RIVPACS, and AUSRIVAS are three of the most widely used multivariate models. (Modified from Reynoldson *et al.* 1997 and Barbour *et al.* 1999).

to change. Conversely, specific functional feeding groups may be particularly susceptible to a specific stressor (e.g., toxic metal uptake by algae would affect grazers). This type of evaluation then relates macroinvertebrate composition to stream processes. These topics are treated extensively by Rosenberg and Resh (1993), Karr and Chu (1999), Barbour *et al.* (1999), Mackie (2001), and Bonada *et al.* (2006).

Biotic indices, which will be used in one of the following exercises, are based on the premise that pollution tolerance differs among various benthic organisms. Tolerance values for each taxon are intended for a single type of pollution, typically for organic (nutrient) pollution, but recently, tolerance scores for certain metals and acidification have been developed (Johnson *et al.* 1993). In most biotic indices, the taxon-specific tolerance value and the abundance (actual, proportional, or categorical) of each taxon in the assemblage are used to calculate a single score using a weighted average approach

(the formula is presented below). Various biotic indices and their formulae are discussed by Metcalfe (1989) and Resh and Jackson (1993).

Tolerance values for individual taxa are derived in a number of ways. One approach is to use collections of benthic invertebrates from streams of varying water quality, and relate the presence or abundance of individual taxa to these conditions. These types of surveys have been published for Wisconsin (Hilsenhoff 1988) and the southeastern United States (Lenat 1993). The more common method of assigning tolerance scores for use in different regions is by modifying previously published tolerance scores by using "expert opinion." Unfortunately, there is circularity in the use of tolerance values in that they are based on where the organisms are found and then applied in an assessment based on an organism's distribution. An inferentially stronger approach would be to base tolerance values on empirically derived laboratory and field testing (Clements 2000, Buchwalter and Luoma 2005). Nevertheless, currently used tolerance values tend to have broad application and often summarize the effects of multiple stressors.

Community similarity indices (e.g., percentage similarity, Bray-Curtis similarity) represent another method for comparing macroinvertebrate composition between sites and are used in both multimetric and multivariate approaches. Most similarity indices compare the composition of two samples on a taxon by taxon basis. These indices often form the basis for cluster and classification analyses as well as advanced multivariate techniques.

The examples of metrics mentioned above are just a small suite of the measurements that can be evaluated when assessing water quality with structural and functional aspects of macroinvertebrate assemblages. An expanded list is presented in Barbour *et al.* (1999, http://www.epa.gov/owow/monitoring/rbp/ch07b.html). Many other metrics (e.g., the proportions of individuals with morphological deformities, fluctuating asymmetry, changes in behavior) have shown promise but are not yet widely used in bioassessments. It should be emphasized that biomonitoring procedures are not static; they continue to evolve as new knowledge becomes available.

B. Multivariate Approaches

Multivariate approaches consider each taxon to be a variable and the presence or abundance of each taxon as an attribute of a site or a time point (Norris and Georges 1993). In contrast to the multimetric approach, the value associated with any given site is a function of that site's taxonomic composition in relationship to the composition of all other sites in the analysis. Multivariate approaches are used more often in large-scale assessments than point-source studies. A variety of procedures are used including many different types of clustering and ordination techniques. Results of clustering and ordination analyses are often combined with other multivariate techniques such as multiple linear regression and discriminant function analysis when relating biological patterns to environmental variables.

Almost concurrently with the onset of the multimetric approach in the United States, the use of multivariate models in biomonitoring began in the United Kingdom (Wright *et al.* 1984). These techniques formed the basis for the development of similar techniques in Canada (Reynoldson *et al.* 1995) and Australia (Davies 2000), and are increasingly being developed and used for biomonitoring in the United States. The initial approach by the United Kingdom led to the development of RIVPACS (**R**iver **In**Vertebrate **P**rediction **A**nd **C**lassification **S**ystem), which is the progenitor of most other multivariate-based biomonitoring approaches used for evaluating streams and rivers. RIVPACS is based on a sequential set of multivariate analyses and is designed to provide site-specific predictions

of the macroinvertebrate fauna to be expected in the absence of major environmental stressors (Wright 2000). The expected fauna is derived by RIVPACS using a database of species presence at reference sites and a small suite of environmental site characteristics. The fauna observed at a site is compared to the model-derived taxa expected at a site (Wright 2000).

Two websites illustrating a multivariate approach that is a derivative of RIVPACS offer detailed presentations about how this approach is being used in wide-scale monitoring programs in Australia: http://ausrivas.canberra.edu.au/Bioassessment/Macroinvertebrates/ Man/Pred/ and http://ausrivas.canberra.edu.au/Bioassessment/Macroinvertebrates/Man/ User/. The application of RIVPACS-type models in the United States can be located at http://129.123.10.240/WMCPortal/DesktopDefault.aspx?tabindex=2&tabid=27.

Multivariate and multimetric approaches can be compared schematically (Figure 35.1). Both approaches require establishing what characteristics (metrics or assemblages) would be typical of unimpaired conditions. Although these approaches differ considerably in the method used for determining whether a test site is equivalent to a reference condition, both methods begin from the same premise and require similar biological data. Models based on metrics and multimetrics generally require greater attention to habitat similarity than some multivariate methods. For example, RIVPACS-type models account for some of the influence of natural habitat variability among sites; however, large reference data sets are necessary for their proper application.

A question that comes up repeatedly in the application of both multimetric and multivariate methods is the level of taxonomy at which macroinvertebrate must be identified for bioassessments. This is a particularly important consideration when evaluating taxon richness (Resh and Unzicker 1975), but is also important regardless of the approach used (Lenat and Resh 1999, Bailey *et al.* 2001). This decision influences many metrics but it is especially critical for richness metrics and the assignment of tolerance values. In some citizens' monitoring programs where volunteer participants depend on "picture keys" to name the organisms collected, identification is usually to a mixture of the order- and family-levels. Because the tolerance of many benthic macroinvertebrates differs within a family and even within a genus (Lenat and Resh 1999), the more detailed the taxonomic resolution the more reliable the assignment of tolerance values. Most state and federal agencies in the USA involved in water quality monitoring typically use a mixture of generic- and species-level identifications (Carter and Resh 2001). In most bioassessments, the level of taxonomy necessary is likely a function of the level of impairment one wishes to detect, with more detailed taxonomy capable of detecting smaller effects.

Habitat Assessment

In the past four decades that benthic macroinvertebrates have been widely used in biomonitoring in North America, there has been a shift in how studies have been conducted. Prior to the early 1970s, emphasis was placed on the use of qualitative sampling with subjective comparisons made to evaluate differences between test and reference areas. Emphasis then shifted to more quantitative studies involving replicated, fixed-area sampling and the use of inferential statistical tests. Importantly, both types of studies were confined to relatively small scales, often point-source type studies. By 1990, emphasis shifted back toward more qualitative sampling approaches and analyses. One reason this latter shift occurred was because of the development and promulgation of Rapid Bioassessment Procedures by the USEPA (e.g., Plafkin *et al.* 1989), which were developed to address larger-scale, nonpoint-source effects. As the spatial scale of studies

has increased, the influence of habitat also has increased and an appreciation for its effects at various geographic scales has developed (Carter *et al.* 1996).

The rationale for including a habitat assessment in a biomonitoring study is that benthic macroinvertebrates are influenced by habitat quality (e.g., bank stability) just as they are by water chemistry (e.g., a pollutant present in the water). Habitat evaluations may have multiple functions. They can be a component of the design of an assessment (as in choosing sites for comparisons), a measure of impact, or a confounding factor in assessment interpretation when habitat and water-quality impairment occur simultaneously. Although visual estimates of habitat quality do not substitute for rigorously measured habitat characteristics in describing and assessing impairment to stream processes that may affect macroinvertebrates (e.g., Chapters 1–6), in many cases they provide an adequate view of general habitat conditions.

When considering water pollution, the relationship between biological and physical condition may best be viewed as:

$$\text{BIOLOGICAL CONDITION} = \text{HABITAT QUALITY} + \text{WATER QUALITY}$$

In the above equation, BIOLOGICAL CONDITION represents a metric (or multimetric) or multivariate measures derived from the macroinvertebrate assemblage, WATER QUALITY represents water chemistry and toxicity, and HABITAT QUALITY represents the geomorphological and biological (e.g., riparian, in-stream algae and macrophytes) conditions at the site.

As important as an analysis of habitat is for confident interpretation of bioassessment results, an overriding consideration in making any physical measurement is to ask *What is the purpose of measuring this characteristic of the lotic environment?* The answer will often determine how much effort and what method should be used for the measurement of each variable chosen. A great deal of effort is often misspent in bioassessments collecting physical measurements that have little to do with the question(s) being addressed. An intensive geomorphological description of a stream reach can take days to years of effort by a large crew of trained fluvial geomorphologists (Fitzpatrick 2001). Restricting the physical evaluation to a suite of variables that are predicted to have a potential influence on macroinvertebrate distributions within the region of the assessment can be extremely efficient (Rankin 1995). Customizing the effort per variable is also useful in reducing total effort (Fend *et al.* 2005). When choosing a specific habitat protocol, it is important to note that many protocols focus on geomorphological influences on channel processes and fish habitat, and do not adequately or specifically evaluate macroinvertebrate habitat.

Prior to beginning any study, appropriate sampling sites must be chosen. The linear dimensions of a site, its reach length, can be based on the geomorphology of the stream (e.g., one pool-riffle sequence; 40× channel widths) or a fixed length of stream (Carter and Resh 2001). Reaches are often chosen in the same stream, for instance above and below a potential source of pollution that is suspected of impairing biological condition in point-source studies. The placement of sites in this manner is a common practice, although it is not an optimal design because upstream and downstream sites could differ in terms of many uncontrolled variables (e.g., discharge could be higher downstream, riparian conditions could differ) and values estimated (mean and variance) using within-site "replicates" would be spatially confounded. Alternatively, sites could be in two or

more different but comparable streams.[1] The inclusion of multiple reaches and better, multiple streams, in the design greatly increases the generality of the conclusions.

The principle that should be remembered when sites are compared is that their physical characteristics should be as similar as possible. These characteristics should include that (1) the gradient of the compared reaches be very similar [e.g., comparison of a high-gradient (5% slope) reach with a meandering (0.5% slope) reach is not meaningful]; (2) the substrate composition or at least the dominant substratum size of each reach be similar (e.g., a sand-dominated channel will have different macroinvertebrates than a cobble-dominated channel); (3) the streams be of similar order and have similar discharge regimes; and (4) the streams be either permanent *or* intermittent (e.g., although an intermittent stream may appear perennial in winter and spring, its invertebrate fauna will be very different than the fauna of a perennial stream). It should also be remembered that sites have "legacies," which could include historical anthropogenic impacts (e.g., early agriculture or logging) and natural (e.g., floods or droughts) events that can greatly influence the fauna present at the time of sampling. Establishing similarity of these factors among studies sites will greatly increase confidence in the resultant assessment.

Once the study reaches have been chosen, individual sampling sites within them must be selected. Frequently, riffles are chosen as sites for macroinvertebrate sampling because of the abundance and diversity of organisms often found in them; however, a combination of riffles and pools or sampling all habitats in proportion to their occurrence also are options. Regardless of the habitat(s) chosen for collecting macroinvertebrates, standardization of the sampling protocol at all sites is critically important for comparisons to be valid.

III. SPECIFIC METHODS

The process of conducting a biological assessment, including a physical habitat assessment of a stream and its surrounding riparian area, can range from a quick estimate of present conditions to detailed measurements made over long periods of time (months or even years). Below, we present methods for rapid biological and physical habitat assessments. The *Basic Method* can be completed during one day in the field and/or one day in the laboratory. This method is particularly appropriate for a survey or reconnaissance study when little is known about a stream, when a spill or pollution problem needs to be evaluated quickly, or for comparisons over large geographic areas within similar environmental settings. Additionally, it is practical for volunteer monitoring groups and student projects. With modification, it can also be applied in establishing pollution control programs in newly industrialized and developing countries (Resh 1995).

The Advanced Method builds on the techniques used in the basic method. It represents a level of effort and analytical sophistication that is more similar to state and national biomonitoring programs. The following exercises cannot begin to cover the full range of techniques and analytical tools available for bioassessments (see also Chapter 36); nevertheless, they provide the foundation for more intensive studies. Reference works

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[1] When conducting these procedures in a class setting, if an impacted stream cannot be compared with a reference stream, then participants could sample macroinvertebrates in distinctly different habitats within one stream (e.g., pools, riffles, vegetated stream margins) or repeatedly sample the same type of habitat within a single stream. Thus, the participants could examine variability in sampling while still learning the concepts and techniques used in biological assessment.

mentioned throughout this chapter and book should be consulted for more intensive studies. Prior to beginning any study, consultation with a statistician and aquatic invertebrate ecologist to develop an experimental design that will adequately address the questions being asked is highly advised.

A. Basic Method: Assessment of Two Sites

Site Selection

Site selection is an important component of all biomonitoring procedures. Because this exercise serves more as a demonstration of biomonitoring approaches, strict adherence to ideal experimental design principles is not necessary. For example, sites can be located on two different (but physically similar) streams or simply in two different reaches on the same stream. Keep in mind that the physical factors listed previously should be similar among all sites analyzed. Try to restrict your study to streams with pools and riffles. If only low gradient, sand-silt bed streams are available for study refer to USEPA (1997) for macroinvertebrate collecting and habitat considerations. Select one site that would be considered a reference site and a second site that is likely to be impaired. Collect an equal number of macroinvertebrate samples (≥ 3) from each site (see following).

Physical Habitat Description

A reasonable on-site description of a stream can be generated by a few simple habitat measures. In most stream studies, these habitat characteristics are nearly always measured and recorded to describe the area under study at the time of sampling: stream width and depth, flow velocity, water temperature, and weather conditions (see Chapters 2–5). Along with recording the geographic location of the site (preferably using a Global Positioning System), these observations provide fundamental information about the size of the stream, what types of organisms might be living there (e.g., some organisms typically live only in cold water, others only in warm water), and summarize the conditions at the time of sampling which can influence sampling efficiency and provide possible explanations when data are analyzed. A simple narrative description of the appearance of the reach, in particular noting the presence of in-stream structures, point-source inputs of effluents, and other features is extremely important.

Which variables are measured depend on the question(s) being addressed by the study and the general conditions of the reach. For example, an investigator might not need to measure dissolved oxygen in a clean, high-gradient mountain stream because it is likely that the water is saturated with dissolved oxygen. However, in a slow-moving, warm stream that may be subject to organic pollution (e.g., sewage), measurements of dissolved oxygen (both during the day and the night) are crucial.

Other characteristics often measured include the gradient (slope), composition of the substratum, mean particle size (as determined by pebble counts; see Chapter 7), stability of the stream banks, the percentage of the stream that is shaded by riparian vegetation, the riparian width and composition, the complexity of microhabitats within the stream, and the number of pieces of large wood present (Platts *et al.* 1983, USDA 1991, Fitzpatrick *et al.* 1998, Barbour *et al.* 1999; see also Chapters 2, 5, and 13). Water-quality measurements such as pH, conductivity, the concentration of dissolved oxygen and nutrients, or the number of enteric bacteria (*E. coli*) present are also often measured (see Chapters 5, 9–11, 14).

The first three measurements below are all related to discharge at the time of sampling (see also Chapter 3 for detailed discussion and methods). These three measurements

should be taken together in a reach having few obstructions and a uniform flow. Record the measurements of physical habitat as they are taken.

1. *Mean Stream Wetted Width*: Measure the width of the stream in meters, from water's edge to water's edge and perpendicular to the flow, for three different transects across the stream. This measurement is dependent on the discharge at that time and the form or shape of the channel.
2. *Mean Stream Depth*: Along the same transects as above, measure the depth (in cm) at 1/4 the distance from the water's edge, again at 1/2 the distance (midstream), and at 3/4 of the way across. Add the 3 values and divide by 4 (divide by 4 to account for the shallow water from the bank edge to the 1/4 distance mark). Record the average depths (in meters) for each transect.
3. *Current Velocity*: Follow the protocols described in Chapter 3. Alternatively, lay a tape measure along the edge of the stream (5–10 m is sufficient). Drop a neutrally buoyant float (e.g., orange or lemon) into the water several meters upstream of where the tape measure begins and measure the amount of time it takes for the float to pass the length of the tape (if the water is very shallow a twig or cork can be used). Repeat 5 times and calculate the average velocity (m/s).
4. *Water Temperature*: Using an electronic thermocouple or thermometer, read the temperature while the probe is still in the water and after a reasonable equilibration period. Always record the time that temperatures are taken. Relatively inexpensive continuous recording thermometers are now widely available (see Chapter 5).
5. *Water Clarity and Quality*: Record whether the water is clear, slightly turbid, or muddy (e.g., whether you can or cannot see the bottom of the stream). Try to note any source of sediment (e.g., storm runoff, construction activities). Also visually evaluate and record whether any oil is apparent and/or whether the water collected has an odor.

Macroinvertebrate Field Collection Option

1. *Sampling Benthic Invertebrates*: Although many devices and techniques are available for collecting aquatic invertebrates (see Chapter 20 and Merritt and Cummins 1996), a D-frame net is most commonly used in macroinvertebrate biotic indicator studies (Carter and Resh 2001). Collect at least three $\sim 0.1\,m^2$ samples per site using a standard 0.33-m wide D-frame kicknet fitted with a 500-µm mesh net. In cobble-to-gravel bottom streams collect macroinvertebrates from riffles. Locate each placement of the kicknet in a random fashion; however, begin collecting at the most downstream location and move in an upstream direction. A video by Resh *et al.* (1990) illustrates how various sampling devices are used. Replication is important for many types of statistical analyses and specific treatments related to benthic macroinvertebrates such as those in Merritt *et al.* (1996) should be consulted.
2. *Field Sample Processing*: Once the sample is collected, macroinvertebrates can either be "picked" in the field or the entire sample can be taken to the laboratory for more controlled processing (see below). If the samples are to be field-picked, place the contents of the net or screen into a large, white-enamel pan with enough water from the stream to cover the invertebrates. Using forceps, an eyedropper, or the "bug spatula" described in Chapter 25, pick out the first 100 invertebrates that you

randomly encounter and place them in a jar with 70% alcohol. Although the tendency will be to pick out the largest organisms, it is essential that all species and size classes are sampled proportionately. Faster-moving organisms will be harder to catch but every effort should be made to sample all taxa evenly.[2] Be aware of organisms in cryptic cases that resemble pieces of substratum or debris (some insects, such as caddisflies, build cases out of natural materials). If one sample does not provide 100 individuals, take one or more additional samples until the total number of invertebrates collected reaches 100. Remember that organisms that are extremely small (e.g., early instars of insects) will often be difficult to identify but are necessary to include if an unbiased sample is desired.

When subsampling is based on a fixed number of individuals it is essential that the same number of individuals be used for comparisons among sites. This is because metrics based on richness (e.g., number of species) may be confounded by the nonlinear relationship between the number of species estimated and the number of individuals examined. A sample of 400 individuals in which 20 species are found cannot be assumed to scale down to a 200-individual sample that contains 10 species. Many protocols recommend that the total number of organisms sorted from the sample be within ±10% of the sorting goal (e.g., 180 to 220 individuals if the goal is 200). However, to our knowledge, the validity of this criterion has not been tested and would make a good follow-up research project.

Many factors influence the effectiveness and comparability of field sample processing, including: mesh size of sampling devices and sieves, "the area sampled," magnification used in sorting, and the time of day and lighting conditions, among others. In general, field sorting is far less reliable than laboratory sorting.

3. *Subsampling*: If a sample contains far more individuals than needed, subsampling is required. This can be done by marking a grid in the bottom of the pan, using random numbers to select individual squares, and then picking out macroinvertebrates from the selected squares. Systematically pick out individuals until a total of 100 have been separated.[3] The important thing to remember about subsampling is that the procedure should not over- or under-represent any particular group.

If the sample is to be taken back to the laboratory for sorting, place the contents of the sample drained of water into an appropriate sized bottle, plastic container, or a sealed plastic bag. Add enough 95% ethanol to cover the contents. The residual water in the sample will produce a final concentration of about 70% ethanol. Label the sample as described below. See Chapter 20 or Moulton *et al.* (2000) for laboratory subsampling and sorting procedures.

4. *Sample Labeling*: Label the sample clearly, giving the date, a clear description of the location including the stream, county, and state, a brief description of the habitat type (e.g., pool, riffle), and the collector's name(s). Write the information on a paper tag using a pencil, and place the tag in the jar with the sample.

5. *Invertebrate Identification*: Either while sorting the sample or after the sample has been picked, separate the macroinvertebrates into groups of similar-looking

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[2] Soda water or club soda can be added to the pan to anesthetize the animals.
[3] A single square will likely have either fewer than 100 or greater than 100 individuals. If all the organisms are sorted from one or more squares, it is likely that >100 organisms will be sorted. However, sort only 100 identifiable organisms because in this exercise the same number of organisms should be used to compare richness among samples and an estimate of density is not necessary.

organisms (i.e., those you think represent a single species or taxon). Use the general key in Chapter 20 to identify an individual from each group to the family level. Record the information on the data sheet provided (Table 35.2). Good general keys for more detailed identifications are available for all benthic macroinvertebrate groups (e.g., Pennak 1989, Thorp and Covich 1991, Smith 2001, Voshell 2003), specific groups such as the insects (e.g., Lehmkuhl 1979, Merritt and Cummins 1996), macroinvertebrates of specific regions (e.g., Clifford 1991), and insects of specific regions (e.g., Usinger 1956, Peckarsky *et al.* 1990). In addition, a video that demonstrates how to use a dichotomous identification key for benthic macroinvertebrates is available (Merritt 2002).

Macroinvertebrate Laboratory-Only Option

If a demonstration (e.g., to a class or volunteer monitoring group) is required because weather conditions or the size of the group do not allow a field visit, the following

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TABLE 35.2 **Form to Record Macroinvertebrate Data.**

DATE: _____

NAME: _____

SITE: _____

A Order/Family	B # of Organisms	C Tolerance Score	D Total
1. _____	_____ ×	_____ =	_____
2. _____	_____ ×	_____ =	_____
3. _____	_____ ×	_____ =	_____
4. _____	_____ ×	_____ =	_____
5. _____	_____ ×	_____ =	_____
6. _____	_____ ×	_____ =	_____
7. _____	_____ ×	_____ =	_____
8. _____	_____ ×	_____ =	_____
9. _____	_____ ×	_____ =	_____
10. _____	_____ ×	_____ =	_____
11. _____	_____ ×	_____ =	_____
12. _____	_____ ×	_____ =	_____
13. _____	_____ ×	_____ =	_____
14. _____	_____ ×	_____ =	_____
15. _____	_____ ×	_____ =	_____
16. _____	_____ ×	_____ =	_____
17. _____	_____ ×	_____ =	_____
18. _____	_____ ×	_____ =	_____
19. _____	_____ ×	_____ =	_____
20. _____	_____ ×	_____ =	_____

Family Biotic Index = Total of Column D divided by Total of Column B = _____

% EPT = Total Ephemeroptera, Trichoptera, and Plecoptera divided by total of
            Column B = _____

Taxa Richness = Total Number of Taxa = _____

activity may be appropriate to illustrate the principles behind biomonitoring with macroinvertebrates.

1. *Laboratory Preparation*: This exercise is intended for demonstration only. The instructor must assemble macroinvertebrates from previous collections or make special collections prior to the laboratory session. Plan to provide at least 100 macroinvertebrates per student. Assemble two "invertebrate soups," one that represents the macroinvertebrate fauna of a reference stream and one that represents that of an impacted stream. To make the "soup," place all the invertebrates that represent the reference site together in a bowl and cover with 70% ethanol. Do the same for the macroinvertebrates from the impacted site.
2. *Sampling*: To take a sample from the "invertebrate soup," swirl the "soup" to evenly distribute the organisms. Then using a tea strainer or a small aquarium net, dip into the "soup" to obtain a sample. The purpose of this is to obtain a random sample of 100 invertebrates. The sample is then placed in a petri dish with ethanol, sorted, and identified as outlined in item 6 of the previous section.

Data Analyses

Just as errors in sampling, sorting, and identification can lead to inappropriate conclusions, errors in data analysis can lead to misinterpretations as well. A systematic approach to each step, from data entry through statistical analysis is necessary for confident data interpretation.

1. *Family Biotic Index*: On the worksheet provided (Table 35.2), list the names of the macroinvertebrate families collected and the number of individuals in each family in the sample. Look up the tolerance score (Appendix 35.1) for each family or higher taxon and write it in the next column; multiply the values in the number column by the tolerance score for that row. Sum the resulting numbers, and then divide this sum by the total number of individuals. Equation 35.1 is used to calculate the *Family Biotic Index* (FBI) (Hilsenhoff 1988):

$$\text{FBI} = \sum_{i=1}^{S} n_i \times t_i \Big/ \sum_{i=1}^{S} n_i \tag{35.1}$$

where n_i and t_i are the number of individuals and the tolerance, respectively, of the ith family and $S =$ the number of families included in the analysis.

The information recorded on Table 35.2 can also be used to calculate several other useful measures (e.g., the total number of families or family richness; percentage of total organisms that are Ephemeroptera, Plecoptera, and Trichoptera (EPT); percentage of total organisms of a particular functional feeding group). Resh and Jackson (1993) and Barbour *et al.* (1999) provide many examples of the various measures that can be calculated. These measures can then be analyzed in the same way outlined below for the FBI. Analyses of FBI values are conducted in

two steps in this exercise: (1) individual participants evaluate their own data from the habitat assessment, calculate a biotic index, and determine the water quality category of the sites selected and (2) within-site and between-site variability is examined by using the data from all participants.

2. *Biotic Index — Analysis of Individual Data*: Water quality can be evaluated using the FBI by comparing the index value calculated from a benthic sample with a predetermined scale of "biological condition." A scale developed for use in Wisconsin to determine the degree of organic pollution is provided in Table 35.3 (Hilsenhoff 1988). For example, find Group A in the sample data set (Table 35.4). An index of 4.5 was calculated from a test stream sample, which would indicate a "good" water quality rating on a scale of "excellent" to "very poor." Find the water quality rating that describes the FBI scores you calculated.

Alternatively, you can assess water quality by comparing a test site to a regional reference condition, or to a "paired" unimpaired site. The similarity (Table 35.5)

TABLE 35.3	Water Quality Based on Family Biotic Index Values from Hilsenhoff (1988).
Family Biotic Index	**Water Quality**
0.00–3.75	Excellent
3.76–4.25	Very good
4.26–5.00	Good
5.01–5.75	Fair
5.76–6.50	Fairly poor
6.51–7.25	Poor
7.26–10.00	Very poor

TABLE 35.4	Sample Data Set for *t*-Test.	
Group	**Reference Site**	**Test Site**
A	3.4	4.5
B	3.2	5.2
C	3.9	6.1
D	5.6	7.9
E	3.1	5.2
F	5.3	5.7
G	4.3	6.5
H	4.3	5.4
I	5.1	6.3
J	3.2	4.7

Summary statistics:

Reference site:
$n_1 = 10 \quad \bar{x}_1 = 4.1 \quad S_1^2 = 0.89$
$\sum x_1 = 41.4 \quad \sum (x^2)_1 = 179.3$

Test site:
$n_2 = 10 \quad \bar{x}_2 = 5.8 \quad S_2^2 = 1.00$
$\sum x_2 = 57.5 \quad \sum (x^2)_2 = 339.6$

TABLE 35.5	Biological Condition Using Percent Similarity of FBI Calculated Between Test-site and Reference-site Samples. Modified from Plafkin *et al.* (1989).
% Similarity	**Biological Condition**
≥ 85%	Unimpaired
84–70%	Slightly impaired
69–50%	Moderately impaired
< 50%	Severely impaired

between the test site and the reference condition can be calculated, and expressed as a percentage as follows:

$$\text{similarity } (\%) = (\text{reference FBI/test FBI}) \times 100 \qquad (35.2)$$

In the example provided, Group A would show a 76% similarity between sites [=(3.4/4.5)×100], indicating that the test site is "slightly impaired" relative to the reference. Calculate the percent similarity between your reference and test site using Equation 35.2, then match this to the water quality thresholds provided in Table 35.5.

3. *Graphical analysis of group data*: Examine the variability of the rapid assessment data. The easiest way to view variability is to graph it. With each point representing a single collection, place each of the sites on the *x*-axis and the calculated measure [e.g., FBI, family richness, % EPT] on the *y*-axis. Mark the predetermined water-quality thresholds directly on this graph and observe the range of water-quality assessments obtained within the same site.

4. *Measure of variability*: One way to compare the variability of metrics (e.g., FBI) is to express the standard deviation of the sample as a percent of the sample mean. This is called the coefficient of variation (CV) and is computed as follows:

$$\text{CV} = (\text{standard deviation/mean}) \times 100 \qquad (35.3)$$

The advantage of calculating the CV is that it has no units. Therefore, it can be used to compare the variability of different types of measures (e.g., % EPT, taxa richness). Barbour *et al.* (1992) provide an example of its use.

B. Advanced Method: Assessment of Multiple Sites

Site Selection

Site selection is an important component of all biomonitoring procedures. In this exercise two groups of sites will be selected — reference sites and putatively impaired sites.

Because this exercise focuses on a larger-scale question than the basic exercise, each site is a replicate of its respective group. An example of a possible impairment to investigate could include the effects of land cover or land use (suburban or urban compared to forested lands). Each group should contain the same number of sites (≥ 5), and sites should be in separate streams if possible. Keep in mind that the factors listed in section IIIA *Physical Habitat Description* should be similar among all sites analyzed. Try to restrict your study to pool-riffle streams. If only low-gradient, sand-silt bed streams are available, refer to USEPA (1997) for macroinvertebrate collecting and habitat considerations.

Macroinvertebrate Collections

Collect a single, composited macroinvertebrate sample from each site using a standard D-frame kicknet fitted with a 500-μm mesh net. Collect from riffles in cobble-to-gravel bottom streams. Locate each of the 10 placements of the kicknet in either a systematic or a random fashion. Each sample should be composed of $10 \sim 0.1$-m^2 collections per site. Carefully clean samples of excess debris and water, place in a container (e.g., quart canning jar), insert a standard collecting label, and fix the sample with ethanol so the final concentration is 70–80%. [If sites are located on low gradient, sand-silt substratum streams collect macroinvertebrates using the "20 jab" method (see USEPA 1997).] Be certain that macroinvertebrate collections are complete before disturbing the study reach as you conduct the habitat assessment.

Habitat Assessment

As mentioned above, the rationale for including a habitat assessment in a biomonitoring study is that benthic macroinvertebrates may be influenced by habitat quality (e.g., bank stability) just as they are by water chemistry (e.g., a pollutant present in the water). Habitat parameters describe components of the stream channel and the surrounding riparian area. Habitat Assessments can be visually based, such as when different conditions for parameters are described verbally or pictorially, scores ascribed to the different conditions, and summations made to describe overall habitat conditions (see Habitat Assessment in the General Design section of this chapter). The most widely used of these is that presented in Barbour *et al.* (1999).

We have prepared a habitat assessment specifically applied to benthic macroinvertebrates that is arranged in a sequence in which characteristics are evaluated from large scale (basin land cover) to in-stream features (Table 35.6). In conducting this assessment, the user judges the condition of these parameters as optimal, suboptimal, marginal, or poor. The variables we included are only a few of many that could be used.

For each parameter, read the description given for each of the four conditions, select the one that most clearly identifies what you observe or know about your basin, and circle the number that corresponds to the condition class. When you have gone through all the parameters, sum the circled values. Determine the habitat condition of both the reference site(s) and the test site(s). These values will be used in later exercises.

TABLE 35.6	Form to Record the Physical Habitat Assessment (based on Petersen 1991, USDA 1998, USEPA 1999, Fend *et al.* 2005). Numbers (20, 15, 10, and 5) refer to the scores to be applied for each condition selected.

Habitat Parameter	Optimal	Suboptimal	Marginal	Poor	Estimated Value
Basin land cover	Near 100% natural	50–75% natural	25–50% natural	0–25% natural	
Score	20	15	10	5	_____
Riparian width[1]	>18 meters	12–18 meters	6–12 meters	<6 meters	
Score	20	15	10	5	_____
Riparian structure and composition	Natural structure with predominantly native vegetation	Natural structure but a high percentage of non-native vegetation	Natural vegetation structure modified and non-native plants predominate	Riparian structure and composition highly disrupted	
Score	20	15	10	5	_____
Shading[2]	>75% of the water surface of sample reach is shaded	50–75% shaded in reach	20–50% shaded in reach	<20% shaded in reach	
Score	20	15	10	5	_____
Channel alteration	Channelization absent or minimal; stream with normal pattern	Slight localized channelization or evidence of historic channelization	40–80% of stream reach channelized	>80% of stream reach channelized	
Score	20	15	10	5	_____
Embeddedness	Boulder, cobble, and gravel particles with obvious open interstices	Boulder, cobble, and gravel particles <25% embedded predominately by sand; negligible silt	Boulder, cobble, and gravel particles 25–50% embedded by a mixture of sand and silt	Boulder, cobble, and gravel particles >50% embedded by sand and silt	
Score	20	15	10	5	_____
Benthic silt cover	No obvious surface deposited silt in the reach	Silt deposits common along stream margins	Interstitial silt extensive in mid-channel	Top surface of substratum silt-covered	
Score	20	15	10	5	_____
Water appearance	Clear and odorless with no oil sheen	Slightly turbid; bottom visible in pools	Turbid; bottom visible in shallow riffles	Very turbid, odiferous, or oily	
Score	20	15	10	5	_____
				Total Score	_____

[1] *Note: natural riparian conditions may lack trees.*
[2] *If the stream is located in a region lacking shaded streams or the stream is >50 m wide, disregard this factor.*

Subsampling, Sorting, and Identification

It is likely that most samples will contain far more than the 300 individuals per sample needed for the exercise and therefore subsampling is required. This can be done by marking a grid in the bottom of the pan, using random numbers to select individual squares, and then picking out all the macroinvertebrates from the selected squares until you have a total of 300 individuals. An inexpensive, more efficient method of subsampling can be found in Moulton *et al.* (2000). Sort all organisms from the subsample(s) using a dissecting microscope set at $10\times$ magnification and identify them to the lowest taxonomic level for which you are confident of your determinations. If you want to distinguish invertebrates to the lowest taxonomic level possible, use operational taxonomic units (e.g., species *a*, species *b*) if the species is unknown and you can accurately categorize the invertebrates you are identifying. Be certain that your identifications are uniformly done among all samples. Input the species × sample data into a spreadsheet. Most statistical and ordination computer programs accept data input from commonly use spreadsheet programs.

Calculate Metrics

Create a list of metrics that you hypothesize will differ between the putatively impaired sites and the reference sites using the list of metrics in Barbour *et al.* 1999. Calculate the value of each metric for each sample.

Comparison of Replicate Samples

We will determine if there is a statistically significant difference between the two sets of sites. A *t*-test can be used to indicate if the two sample means are the same or if they are significantly different from a statistical point-of-view. To do this test, calculate the mean ($\bar{x}$), variance (s^2), sum of observations ($\sum x_i$), sum of squared observations ($\sum x_i^2$), and the number of observations for each site (n) using samples as replicate observations (see Sokal and Rohlf 1995 or Zar 1999). An example is provided in Table 35.4 (see Narf 1985).

A *t*-test is used to choose between two hypotheses regarding the two populations that were sampled. The null hypothesis (H_0) is that the two population means are equal. The alternative hypothesis (H_A) is that the two means come from different populations. The *t*-test actually tells us the probability (p) that the null hypothesis (H_0) is true (i.e., the probability that $mean_1 = mean_2$). By convention, when the probability is less than 1 in 20 (i.e., $p < 0.05$) the two means are considered to be significantly different. To calculate the *t*-statistic, take the difference between the sample means and divide by the standard error of this difference, as shown by the following equation:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{S^2_{n_1+n_2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \tag{35.4}$$

where the means and sample sizes are taken directly from the summary statistics. The pooled variance $s^2_{n_1+n_2}$ is calculated as follows:

$$S^2_{n_1+n_2} = \frac{\left(\sum_{i=1}^{n_1} x_i^2 - \bar{x}_1 \sum_{i=1}^{n_1} x_i\right)_1 + \left(\sum_{i=1}^{n_2} x_i^2 - \bar{x}_2 \sum_{i=1}^{n_2} x_i\right)_2}{n_1 + n_2 - 2} \qquad (35.5)$$

A computed t-test using the sample data in Table 35.4 is:

$$t = \frac{5.75 - 4.14}{\sqrt{0.94 \left(\frac{1}{10} + \frac{1}{10}\right)}} = \frac{1.61}{0.43} = 3.74 \qquad (35.6)$$

The critical value for t (t_{crit}) is then looked up in a t-table. The t_{crit} depends on the number of samples used to calculate t. The data above have a total of 20 samples, and therefore 18 degrees of freedom (df); where df $= n_1 + n_2 - 2$. For 18 df, the $t_{crit\ (0.05)}$ $= 2.10$ (Table 35.7). Our calculated t is greater than this critical t-value, and therefore we conclude that the two means are significantly different ($p < 0.05$). (If the calculated t is negative, use the absolute value.) A published t-table should be consulted for critical values (e.g., Sokal and Rohlf 1995, Zar 1999) but Table 35.7 contains some 2-tailed $t_{crit\ (0.05)}$ values for sample sizes ($N = n_1 + n_2$) ranging from 7 to 37 (df $-5-35$).

The above procedure can be used to evaluate many different types of measures (e.g., those listed earlier). Note that the t-test assumes that both samples come from a normally distributed population and that the variances (s^2) of both samples are equal. When sample sizes are equal, these assumptions may be "relaxed." However, it is always a good idea to know if these assumptions are being met. A quick rule-of-thumb to check for equal variances is to make sure the ratio of the sample variances is less than 2. If this ratio is

TABLE 35.7	List of Two-tailed Critical *t*-Values (p < 0.05) for Various Degrees of Freedom (df). Use the next smaller N if your sample size is between the values provided, or consult a more extensive *t*-table (e.g., Zar 1999).

N	df	Critical *t*
7	5	2.57
12	10	2.23
17	15	2.13
22	20	2.09
27	25	2.06
32	30	2.04
42	40	2.02

greater than 2, then you may need to transform the data or use a test that does not assume equal variance. On the other hand, if the variances differ, the stressors may be affecting the variability of the metric rather than the mean value. Refer to a statistical text (e.g., Sokal and Rohlf 1995, Zar 1999) for further information on these alternatives. Always be confident that the various assumptions for parametric statistics are met before drawing a conclusion based on the test.

Generate Ordination Scores

Using one of the ecology-based statistical packages such as PC-ORD (McCune and Mefford 1999), ordinate your species × sample data using the indirect-gradient analysis method of Detrended Correspondence Analysis (DCA) choosing default program settings. We suggest using DCA instead of Non-metric Multidimensional Scaling (NMDS) because in our experience single physical variables are usually more highly correlated with the site scores of the first axis of a DCA than with NMDS axes. Save the site scores from at least the first axis for use in the next step.

Correlations

Using an available statistical program correlate the above generated site scores from the DCA and selected metrics with the per-site index of habitat condition calculated under *Habitat Assessment* above. To further explain these relationships calculate the correlations between the multivariate site score and selected metrics with individual habitat variables. Plot each correlation.

IV. QUESTIONS

General Questions for Both Methods

1. Choose two physical parameters and describe what the optimal condition would be and what the poor condition would be in reference to the abundance and distribution of macroinvertebrates. Would these optimal values be the same for other stream organisms such as fish, amphibians, or algae?
2. You are planning to conduct a biomonitoring comparison between two streams — one presumed to be pristine, the other impacted. How would you avoid the possibility of "uncontrolled variables" influencing your results? That is, account in your experimental design for factors that would indicate impairment when no impairment has actually occurred.
3. You have been placed in charge of a team to monitor urban streams. What type of impacts might be present and how would you design a biomonitoring study that would detect these impacts?
4. Oftentimes, only a single measurement of a physical (e.g., temperature) or a water chemistry (e.g., conductivity) variable is taken at a site; however, we often take replicate biological measures because we assume that intersample variability will occur. Was the biological variability greater than the variability in physical and water chemistry parameters you measured?
5. How do you think that physical, chemical, and biological measurements would vary in streams in your region throughout the year? How would numerical values of benthic macroinvertebrate metrics vary throughout the year?
6. What types of legacy (i.e., effects that occurred previously at a site or in a basin) effects could influence the results of a biomonitoring survey?

Questions for Basic Method

7. In Table 35.3, Family Biotic Index scores are ranked into category ranges that denote specific water-quality judgments (e.g. Excellent, Good, Poor). Are there any caveats you would warn the public about in using these judgments based on FBI scores that you calculated from your samples?
8. If several samples were taken from each stream, how similar were the FBI scores within a stream? Do you think you would get different FBI scores if you looked in different habitats (e.g., riffles, pools, stream edges, vegetation)? How could the variability among samples within a stream been reduced?
9. If you sampled a reference stream and an impaired stream, was the variability among samples the same in each stream? Do you think the variability among samples should be higher in a reference stream or an impaired stream?

Questions for Advanced Method

10. What are the strengths and weaknesses of visually based habitat assessment approaches?
11. In reviewing quantitative approaches to physical habitat assessments in streams (Chapters 1–6 in this book), which have potential applications in interpreting results of biomonitoring programs?
12. Metrics are presumed to be based on accepted ecological theory. Does the expectation of a decline in species richness with increased impairment reflect the predictions of the Intermediate Disturbance Hypothesis, the ecological theory on which the predicted response of this metric is based?
13. Your results were based on identifying macroinvertebrates to the lowest taxonomic level possible. Do you think your conclusions would be different if identification was only to the family level? Why or why not?
14. Were metrics statistically different between the two groups of sites? Could you sample fewer sites and still detect a difference for these metrics?
15. Were there significant correlations between the physical variables and the site scores? If so, how would you incorporate this information into designing a larger-scale bioassessments?

V. MATERIALS AND SUPPLIES

Maps showing collecting site locations and land cover
Global Positioning System unit
Calculator
Computer software (e.g., spreadsheet, ecological)
Dissecting microscopes
Enamel pans
Equipment for collection of macroinvertebrates (see Chapter 20)
Equipment to measure current velocity (see Chapter 3)
Ethanol (95% and 70%)
Forceps (fine-tipped)
Keys to identify macroinvertebrates (e.g., Appendix 20.1; Merritt and Cummins 1996)

Petri dishes to sort samples
Thermometer
Vials, jars, or sealable plastic bags

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APPENDIX 35.1
Tolerance Values for Macroinvertebrates

(most from Hilsenhoff 1988;
*adapted from Lenat 1993,
**from Bode 1988)

Plecoptera

Capniidae	1
Chloroperlidae	1
Leuctridae	0
Nemouridae	2
Perlidae	1
Perlodidae	2
Pteronarcyidae	0
Taeniopterygidae	2

Ephemeroptera

Baetidae	4
Baetiscidae	3
Caenidae	7
Ephemerellidae	1
Ephemeridae	4
Heptageniidae	4
Leptophlebiidae	2
Metretopodidae	2
Oligoneuriidae	2
Polymitarcyidae	2
Potamanthidae	4
Siphlonuridae	7
Tricorythidae	4

Odonata

Aeshnidae	3
Calopterygidae	5
Coenagrionidae	9
Cordulegastridae	3
Corduliidae	5
Gomphidae	1
Lestidae	9
Libellulidae	9
Macromiidae	3

Trichoptera

Brachycentridae	1
Calamoceratidae*	3
Glossosomatidae	0
Helicopsychidae	3
Hydropsychidae	4
Hydroptilidae	4
Lepidostomatidae	1
Leptoceridae	4
Limnephilidae	4
Molannidae	6
Odontoceridae	0
Philopotamidae	3
Phryganeidae	4
Polycentropodidae	6

Psychomyiidae	2
Rhyacophilidae	0
Sericostomatidae	3
Uenoidae	3

Megaloptera

Corydalidae	0
Sialidae	4

Lepidoptera

Pyralidae	5

Coleoptera

Dryopidae	5
Elmidae	4
Psephenidae	4

Diptera

Athericidae	2
Blephariceridae	0
Ceratopogonidae	6
Blood-red Chironomidae	8
Other Chironomidae	6
Dolichopodidae	4
Empididae	6
Ephydridae	6
Psychodidae	10
Simuliidae	6
Muscidae	6
Syrphidae	10
Tabanidae	6
Tipulidae	3

Amphipoda**

Gammaridae	4
Hyalellidae	8

Isopoda**

Asellidae	8

Acariformes** 4

Decapoda**

Astacidae	6

Gastropoda**

Lymnaeidae	6
Physidae	8

Pelecypoda

Pisidiidae	8

Oligochaeta** 8

Hirudinea** 10

Turbellaria** 4

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Establishing Cause-Effect Relationships in Multi-Stressor Environments

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I. INTRODUCTION

Pollution effects in riverine environments are seldom the result of single stressors. This is because flowing waters receive multiple, and potentially interacting, effluent discharges from municipalities and industries, and diffuse inputs from non-point sources (e.g., agriculture). This reality makes it difficult to establish cause-and-effect relationships through standard field biomonitoring of rivers, particularly given that the duration and concentration of effluent exposure is often poorly described. Adequate replication of stressor concentration along pollution gradients (e.g., effluent plumes) can also be difficult to achieve because spatial heterogeneity of the benthic environment is frequently confounded within field sites (Glozier *et al.* 2002). Recent expert reviews of monitoring programs in Canada concluded that unsuccessful field assessments were linked to the presence of multiple effluent discharges, interaction of contemporary effluent stressors with legacy effects of past pollution, and uncertainties regarding exposure to effluents (Megraw *et al.* 1997).

Stressor effects can be cumulative and interact across varying spatial and temporal scales, and thus the process of defining causal relationships is highly complex. Culp *et al.*

(2000a) identify three different categories of impacts that complicate the establishment of causal relationships in river ecosystems. *Incremental impacts* represent the additive effect of similar stressor events whose combined effect exceeds a critical ecological threshold. *Multiple source impacts* occur when sources of stressors and their effects overlap spatially. *Multiple stressor impacts* include scenarios where different classes of stressors interact in a synergistic or antagonistic fashion preventing *a priori* prediction of biotic responses. In this chapter we focus on a combination of methodologies for investigating multiple stressor effects of effluent discharges.

Adams (2003) recently evaluated the wide variety of approaches that researchers have implemented to establish cause-and-effect relationships between environmental stressors and biological response variables (i.e., response endpoints). The broad categories identified include laboratory toxicity tests, field bioassessments, field experiments, simulation modeling, and hybrid methodologies that combine aspects of two or more approaches. The integration of laboratory, field, and experimental manipulation studies is particularly useful for establishing causality and has been employed to assess the impact of heavy metals (Clements and Kiffney 1994) and pulp mill effluents (Culp *et al.* 2000b) on the benthos of rivers. These integrated approaches have the advantage of using field observations to focus hypothesis generation for experimental studies that identify cause-and-effect relationships.

Establishment of strong causal linkages between stressors and biological responses is facilitated by integrating information from a variety of sources through weight-of-evidence methodologies (Lowell *et al.* 2000). This concept incorporates an eco-epidemiological approach that evaluates the strength of the causal relationship by using a formalized set of criteria developed previously in the field of epidemiology. Several authors (Fox 1991, Suter 1993, Gilbertson 1997, Beyers 1998, Lowell *et al.* 2000) have forwarded weight-of-evidence postulates that provide logical guidelines for establishing causation in ecological risk assessment, and these postulates have recently been incorporated by the USEPA in a stressor identification protocol (USEPA 2000). The multiple criteria proposed by various authors have been reduced to seven assembly rules that can be consistently applied in studies of ecological risk assessment (Adams 2003). These criteria are outlined in Table 36.1 and include (1) strength of association; (2) consistency of association; (3) specificity of association; (4) time order or temporality; (5) biological gradient; (6) experimental evidence; and (7) biological plausibility. Essentially, this method requires that biological effects be associated with stressor exposure, plausible mechanisms that link cause and effect, and experimental verification of causality that is in concordance with available field evidence. Lowell *et al.* (2000) suggest that it may be useful to assign weights to each criterion in order to establish their relative importance if not all of the criteria are satisfied, or when the findings for some criteria conflict with others. Studies that include the methods described in this chapter will produce the baseline information needed for application of this eco-epidemiological approach to assess ecological risk of an effluent discharge.

A. Linking Field Biomonitoring to *In Situ* Bioassay Experiments

Field biomonitoring is an important component of contemporary impact assessment because field surveys can identify the biological responses to pollution. Nevertheless, field surveys cannot easily link cause and effect (Adams 2003), which is why we combine biological assessment with field experiments. Most industrial and municipal effluents contain an array of compounds and their effects on aquatic organisms can be stimulatory

TABLE 36.1	Formalized Set of Causal Criteria Forming Part of a Weight-of-Evidence Approach for Ecological Risk Assessment. Causal criteria are from Adams (2003).
Causal Criterion	**Support for Criterion**
Strength of association	Cause and effect coincide. Many individuals are affected in the exposure relative to the reference area.
Consistency of association	The association between a particular stressor or stressors and an effect has been observed by other investigators in similar studies at other times and places.
Specificity of association	The effect is diagnostic of exposure.
Time order or temporality	The cause precedes the effect in time and the effect decreases when the cause is decreased or removed.
Biological gradient	There is a dose-response relationship either spatially or temporally within the system.
Experimental evidence	Experimental studies support the proposed cause-and-effect relationship.
Biological plausibility	There is a credible biological and/or toxicological basis for the hypothesized mechanism linking the proposed cause and effect.

as a result of nutrient enrichment, or inhibitory because of contaminant toxicity. *In situ* bioassays are useful tools in this regard as their application can help researchers tease apart the ecological effects of nutrients from those of contaminants. In this way, the confounding factors of multiple stressors can be isolated and measured so that the cause of biological responses downstream of an effluent discharge is revealed.

Nutrient-diffusing substrates (NDS) are an ideal method for establishing the effects of effluents on nutrient limitation (see also Chapter 10). Chambers *et al.* (2000) used NDS experiments to demonstrate that periphyton biomass was maintained at low levels by insufficient P upstream of point-source discharges, while effluent loading from pulp mill and sewage inputs alleviated nutrient limitation downstream of major discharges. Furthermore, they deployed NDS bioassays throughout river basins to draw broad conclusions about the cumulative effects of effluent discharges on algal standing crop (Scrimgeour and Chambers 2000). Similarly, by performing NDS experiments in the autumn and winter, Dubé *et al.* (1997) demonstrated that the effects of nutrient additions from effluents varied seasonally. In both studies, the researchers developed their experimental hypotheses based on patterns observed initially in field survey results.

Field-based, toxicity bioassays are not a new approach — for centuries, miners used caged birds to detect the presence of carbon monoxide in tunnels. However, the use of caged organisms to detect aquatic pollution is a more recent development, and these methods are more sophisticated, involving the measurement of sublethal endpoints such as feeding behavior and growth, in addition to mortality (Burton *et al.* 2001, McWilliam and Baird 2002a). These *in situ* bioassays employ a wide variety of organisms, and can provide useful insight into the ability of animals to perform their functional role within the ecosystem. McWilliam and Baird (2002a) distinguish two classes of these bioassays based on the timing and location of the response endpoint determination: *exposure bioassays*, where the measurement is made at the same time as the field exposure; and *postexposure bioassays*, where the measurement is made at some time after the field exposure (often immediately afterward). These two approaches have contrasting

advantages and disadvantages. Exposure bioassays allow the signal strength of any effect to be maximized (as the animal is performing under stress), although the signal is also accompanied by environmental noise (i.e., from environmental factors varying within a natural range such as temperature), which can often mask the signal, and confound differences across sites. In contrast, postexposure bioassays are based on the principle that certain stress responses will persist following the removal from the stressor source, with sublethal toxicity being a good example of this (Taylor *et al.* 1998). Postexposure bioassays, therefore, can separate natural environmental stress factors from anthropogenic factors, and are particularly useful for determining the presence of bioavailable toxic substances. In this chapter, we describe the use of a postexposure bioassay that integrates mortality and feeding rate responses to evaluate the toxicity response of biota to effluent discharge.

B. Artificial Stream Approaches

In contrast to field biomonitoring or *in situ* bioassays, artificial stream experiments can control relevant variables and help isolate potential agents, such as nutrients or contaminants that cause the biological response. This research tool incorporates greater ecological complexity than is possible to include in laboratory toxicity tests, and can generate important information on the chronic effects of pollutants on riverine communities. Stream mesocosms (i.e., artificial streams) vary widely in design, from simple laboratory systems to elaborate outdoor complexes (Lamberti and Steinman 1993), and have been used to examine many levels of biological organization, ranging from single species to multispecies tests (Guckert 1993). The use and application of stream mesocosms in ecotoxicology has been reviewed by many authors over the last 30 years (Shriner and Gregory 1984, Kosinski 1989; Guckert 1993; Pontasch 1995; Rodgers *et al.* 1996; Culp *et al.* 2000d). Integration of stream mesocosm studies with field biomonitoring is a particularly beneficial approach for retrospective ecological risk assessments (Suter 1993) and has be used to generate weight-of-evidence risk assessments for large rivers (Culp *et al.* 2000b). In this chapter we combine artificial stream use with field biomonitoring and *in situ* bioassays to better understand the relationship between exposure to specific pollutant sources and the resulting ecological consequences.

The specific objectives of this chapter are to (1) outline a basic methodology for assessing the biological responses to an effluent discharge (i.e., a point source); (2) demonstrate how NDS bioassays can be used to evaluate the effect of effluents on nutrient limitation of algal biomass; (3) illustrate the usefulness of field-based toxicity bioassays to determine contaminant effects of effluents; and (4) introduce the use of stream mesocosms as an approach to establish causality between stressors and biological effects. Together, the different methods provide a process through which key stressors are identified, ecological effects are measured, and subsequent investigations into the cause of effects are conducted.

II. GENERAL DESIGN

This chapter describes methods and a conceptual framework for evaluating the effects on benthic communities of an effluent discharge that contains both nutrients and contaminants. Environmental assessment proceeds sequentially from basic to advanced approaches such that studies that complete the sequence will be able to use weight-of-evidence criteria to draw conclusions about the cause of the measured biological effects.

The tiered approach follows a decision tree of logic similar to the Canadian Environmental Effects Monitoring program for metal mining (Glozier *et al.* 2002) and methods of causal analysis outlined by Burton *et al.* (2001).

Under this approach, the questions posed within each assessment tier become progressively more focused with the ultimate goal of identifying the cause of the measured biological effects. First, the existing information for the effluent exposure site is summarized including the identification of potential reference and exposure areas, review of historical data on water quality and effluent composition, and consideration of existing benthic invertebrate data. The effluent constituents are characterized, key stressors are identified, and effluent dilution in the exposure area is estimated. The second tier determines if there is an effect on the benthic invertebrate community within the immediate vicinity of the effluent discharge. The most likely cause of the ecological effects is determined by comparing the ecological effects data with the list of major stressors in the effluent. Within this assessment tier the research can also identify the spatial extent of the effect by locating additional exposure sites downstream. The third tier aims to produce mechanistic understanding of the responses of the benthic invertebrate community to the key stressors by conducting one or more field experiments chosen from a wide variety of available assessment tools. The application of explicit weight-of-evidence criteria to the information collected within each tier will strengthen cause-and-effect understanding of stressor and ecological effects relationships.

A. Site Selection

Third- to fourth-order streams, which have cobble and pebble substrate, and that are wadeable are ideal for the application of these methods. However, the approach is also appropriate for larger rivers when riffle areas along the shoreline can be safely waded. Reference and exposure habitats must be comparable if the ecological effects of the effluent stressors are to be separated from natural habitat variability. The reference area should be free from effluent exposure. Care must be taken to choose reference and effluent-exposure sites with similar habitat features including stream channel geometry (i.e., bankfull width and depth, channel gradient; see Chapter 4), substrate particle size, current velocity, discharge (see Chapter 3), and riparian vegetation.

Your choice to examine the ecological effects on the benthic community of a particular effluent discharge will be based on the assumption that the effluent contains potentially deleterious contaminants or nutrients that may cause unacceptable environmental effects. Your assumption can be strengthened through qualitative observations such as changes below the effluent discharge in plant biomass, the composition of the benthic community, or records of acute events such as fish kills. In the absence of such observations, you can choose to examine the effects of a particular discharge based on public concern that the point source is causing ecosystem impairment. Regardless of the pathway of problem initiation, the objective of the ecological assessment will be to determine if the effluent discharge is having an effect on the benthic invertebrate community (measured as a statistical difference between the reference and exposure areas in a control versus impact study design).

B. General Procedures

Ecological effects of the effluent discharge for the *Basic Method* will be assessed by changes in algal biomass (measured as chlorophyll *a*) and several benthic invertebrate

endpoints (i.e., total numerical abundance, taxonomic richness, evenness, Bray-Curtis index). Most field sampling techniques and laboratory analyses incorporated in the basic and advanced methods are described in detail elsewhere in this book. Methods for the collection and processing of benthic invertebrate samples are fully described in Chapter 20. Sample collection and laboratory processing of chlorophyll *a* is detailed in Chapter 17, while water collection and analytical methods for nutrient analysis (N, P) are covered in Chapters 9 and 10. Analysis of water samples for general ions and metals, and analysis of the full-strength effluent for nutrients and contaminants (i.e., metals, acids and derivatives, phenols, alcohols, aldehydes and ketones, hydrocarbons) will best be done in a commercial lab following methods outlined in APHA *et al.* (1995).

Procedures and experimental approaches used in the Advanced Methods will vary depending on the researcher's needs. However, by combining two or more of the techniques, you will be able to assign any measured effects to the presence of nutrients or contaminants in the effluent. The nutrient-diffusing substrate technique (Tank and Dodds 2003) can be used to detect whether the effluent modifies nutrient limitation in the river. We use *in situ* bioassays of feeding rate to measure sublethal toxicity effects. Artificial stream techniques are excellent methods for establishing cause-and-effect relationships as the researcher can control relevant environmental variables and separate the effects of multiple stressors in the effluent (e.g., nutrient versus contaminant effects).

III. SPECIFIC METHODS

A. Basic Method 1: Retrospective Ecological Risk Assessment of an Effluent Discharge

This method employs a conceptual framework for assessing environmental effects that is modified from Burton *et al.* (2001) and Glozier *et al.* (2002). The approach evaluates existing databases to help focus the assessment and analyzes effluent composition to identify possible nutrient and contaminant stressors of concern. Following this sequence of problem definition, site characterization, and identification of potentially important stressors, the method extends to assessment of ecological effects by comparing benthic communities at reference and exposure sites. Such field assessments can adequately measure environmental impacts on biological communities and, further, can suggest a potential cause of the ecological effect (e.g., excessive nutrient addition). Nevertheless, it is difficult to establish cause-and-effect relationships unequivocally with this type of field bioassessment design because the approach relies on statistical inference (Stewart-Oaten *et al.* 1992, Cooper and Barmuta 1993). For example, the limited knowledge of the concentration and duration of stressor exposure and excessive spatial heterogeneity complicates the assignment of causality. The advanced methods below provide further approaches that produce information to link cause with ecological effect.

The primary objective of the ecological assessment will be to assess quantitatively if the effluent discharge is having an effect on the benthic invertebrate community. This type of effects examination is termed *retrospective ecological risk assessment* because the effluent pollution began in the past and likely has ongoing consequences (Suter 1993). Effects will be measured as a statistical difference between the reference and exposure areas in a control versus impact study design. You must complete the steps of site characterization, field assessment of ecological effects, and data interpretation to complete the basic retrospective effects assessment.

Site Characterization

A principal role of the site characterization step is to gather sufficient information to identify potential exposure and reference areas that have similar habitat characteristics (Table 36.2). The five steps of site characterization require you to collect habitat information for the sites, assess the quality of previous benthic invertebrate data, estimate the concentration of effluent in the exposure area, examine the effluent constituents, and produce a list of stressors that may cause ecological impairment.

1. Collect information on the channel geometry (i.e., bankfull width and depth, channel gradient) and substrate particle size following the protocols in Chapter 4. Measure the current velocity and discharge at the potential sample sites following methods in Chapter 3. Note any other inputs to the exposure or reference areas (e.g., storm water, sewer outfalls). Identify and describe all other factors, either anthropogenic or natural (e.g., riparian vegetation), that are not related to the effluent under study, and that might confound the comparison of observed differences in effects measures between the reference and exposure sites. Much of this information may be available in government reports or other public domain sources that describe earlier environmental assessments of the discharge. Briefly summarize the production processes that contribute to the effluent source (e.g., type of industrial facility), any effluent treatment processes employed by the discharger, and the mean daily amount of effluent discharged to the receiving water. Use the above information to justify your choice of a reference site above the influence of the effluent discharge and an exposure site 500–1000 m below the effluent outfall. In this example you will use a simple, *Control versus Impact* study design. However, more complicated designs, which incorporate additional reference and exposure sites, are available (Glozier *et al.* 2002).
2. Summarize the available benthic invertebrate community data collected during previous environmental assessments. Determine the adequacy of the historical data

TABLE 36.2	Site Characterization Information for the Retrospective Assessment of a Point Source Discharge.
Information Category	**Reference and Exposure Site Descriptors**
Physical characteristics	Reach gradient; substrate particle size composition; mean annual discharge and range; mean annual water temperature and range
Effluent treatment	Summary of effluent type and description of the treatment process
Effluent constituents	Summary of the major constituents of the effluent including nutrients, general ions, metals, and contaminants (i.e., acids and derivatives, phenols, alcohols, aldehydes, ketones, hydrocarbons)
Effluent mixing	General description of how the focal effluent mixes with the receiving water with a semiquantitative or quantitative estimate of effluent concentration at the exposure site
Other effluent discharges	Location and description of other effluent discharges (e.g., stormwater outfalls)
Species at risk	Summary of any rare, threatened, or endangered aquatic species in the study area

set by assessing the quality assurance and quality control methods employed for the field and laboratory procedures. This review will list the sampling device and mesh size used in the earlier study, and determine whether the study design and replication was appropriate. Assess whether the quantity or physico-chemical quality of the discharge has changed since the previous study. If the data sets are of good quality and the effluent discharge is unchanged, then these data can be used to focus your study question to the Advanced Method. If historical benthic invertebrate data do not exist or is of poor quality, or the effluent has changed substantially, then you will conduct a field assessment following the protocol described below.

3. Estimate the concentration of effluent at the exposure site area by conducting a conductivity survey in the field using a standard conductivity meter. This survey will measure the conductivity in the full-strength effluent (C_e), at the reference site upstream of the effluent (C_u), and at the downstream exposure site (C_d). Conductivity measurements from the field survey can be converted into relative effluent concentrations (C_r) ranging from 1 (full-strength effluent) to 0 (background) by applying the following formula:

$$C_r = \frac{C_d - C_u}{C_e - C_u} \qquad (36.1)$$

where C_e is effluent conductivity (μS/cm); C_u is reference site conductivity (μS/cm); C_d is exposure site conductivity (μS/cm); and C_r is the relative concentration (i.e., dilution ratio) of the effluent at the exposure site. If a conductivity survey is not undertaken, an indication of effluent concentration at the point of complete mixing can be estimated by dividing the mean daily discharge of effluent by mean daily stream discharge during the period proposed for the benthic invertebrate survey.

4. Collect a sample of the full-strength effluent and analyze the sample for major ions, nutrients, and contaminants (i.e., metals, acids and derivatives, phenols, alcohols, aldehydes and ketones, hydrocarbons). Requisite sample volume is typically at least 1 L and replicate water samples are advised. Effluent chemistry is best done in a commercial lab following methods outlined in APHA *et al.* (1995).

5. Make a candidate list of the nutrient and contaminant stressors that are most likely to cause impairment in the benthic invertebrate community. Estimate the stressor concentration (S_c) in the exposure environment using the following formula:

$$S_c = C_r \times S_i \qquad (36.2)$$

where C_r is the dilution ratio; S_i is the concentration of the nutrient or contaminant stressor in the full-strength effluent; and S_c is the estimated concentration of the stressor at the exposure site. Compare the estimates for the environmental concentration of nutrients and contaminants to recommended water quality guidelines (e.g., CCME 1999; USEPA 2002). S_c estimates that exceed these

guidelines should be considered as contributors to potential causes of impairment. Further discussion on identification of stressors can be found in USEPA (2000).

Field Assessment of Ecological Effects

1. Collect 5 replicate samples of benthic invertebrates from the reference and exposure sites following the quantitative methods outlined in Chapter 20. Transfer the samples from the collection net to a storage container that is clearly labeled in pencil (site, replicate number, date, sample collector's name). Preserve the sample in 95% ethanol (final concentration in container of $\sim$70% ethanol). Place permanent paper labels (i.e., written in pencil) inside the sample container. It is preferable to process samples for retrospective analysis in the laboratory (see Chapter 35).
2. Collect 5 replicate periphyton samples from cobble substrate by using a scalpel to scrape the periphyton from within a $10\,cm^2$ area. Caps from 50 mL centrifuge tubes are ideal templates for delimiting the sample area, either by etching the rock with a pencil or by scraping away the periphyton from outside the cap to reveal the sampling area. Other simple quantitative samplers are described in Chapter 17. Transfer the periphyton sample to a labeled vial or bag, and store the samples on ice in the dark until they can be processed in the laboratory (preferably within 4 h).
3. Collect water samples for estimates of major ions and nutrients, store the samples on ice, and analyze in the laboratory (nutrients) or ship immediately to an analytical laboratory (major ions) for processing.

Laboratory Methods

1. Follow the methods of Chapter 35 to sort and identify the benthic invertebrates to the taxonomic level of family. Record the abundance of each family for each sample.
2. Determine chlorophyll *a* abundance for each periphyton sample using the protocol in Chapter 17.
3. Process water samples according to methods in APHA *et al.* (1995).

Data Analysis

1. Calculate the total invertebrate density (i.e., total number of individuals in all taxa) for each sample expressed per unit area (numbers/m^2).
2. Calculate the taxonomic richness (i.e., total number of different taxa) for each sample (i.e., family richness/unit area sampled).
3. Calculate Evenness (E) for each sample as:

$$E = 1 / \sum_{i=1}^{S} (pi)^2 / S \qquad (36.3)$$

where p_i is the proportion of the ith taxon in the sample, and S is the total number of taxa in the sample.

4. Calculate the Bray-Curtis (B-C) Index to estimate the dissimilarity of each sample from the median taxonomic density at the reference site. The B-C Index is a distance coefficient that reaches a maximum value of 1 for two sites that are entirely different and a minimum value of 0 for two sites that possess identical taxonomic composition. The B-C Index measures the difference between sites and this is calculated as:

$$B\text{-}C = \frac{\sum_{i=1}^{S} |y_{in} - y_{ir}|}{\sum_{i=1}^{S} (y_{in} + y_{ir})} \qquad (36.4)$$

where $B\text{-}C$ is the Bray-Curtis distance between sample n and the reference median; y_{in} is the count for taxon i at site n; y_{ir} is the median count for taxon i at the reference site; and S is the total number of taxa present at site n and the reference sites. Tables 36.3–36.5 illustrate the steps for calculating the Bray-Curtis distance (B-C) between each sample and the median taxon density at the reference site. First, determine the median taxon density at the reference site (see Table 36.3). A similar table is constructed for the exposure stations without the median calculation. Calculate the distances between each sample and the reference median following the example illustrated in Table 36.4 (repeat this procedure for all reference and exposure site samples). For this approach, the reference median for

TABLE 36.3 Data Forms for Taxon Density at Reference and Exposure Sites. (A) Example of the determination of the median taxon density at the reference site from a study design with five replicate samples. (B) Example of taxon densities at the exposure site without the median calculation. Example is modified from Environment Canada (2002).

A	Taxon Density (numbers/m^2)				
Reference Sample Number	Taxon 1	Taxon 2	Taxon 3	Taxon 4	Taxon 5
Reference 1	2	3	2	3	1
Reference 2	3	5	2	4	3
Reference 3	9	1	1	1	1
Reference 4	4	6	3	4	1
Reference 5	5	4	2	3	2
Reference Site Median Value	4	4	2	3	1

B	Taxon Density (numbers/m^2)				
Exposure Sample Number	Taxon 1	Taxon 2	Taxon 3	Taxon 4	Taxon 5
Exposure 1	23	4	2	10	1
Exposure 2	12	2	2	8	3
Exposure 3	14	6	1	6	2
Exposure 4	13	1	3	12	2
Exposure 5	15	3	2	4	1

TABLE 36.4	Example Calculation of Bray-Curtis (B-C) Index for a Reference Sample. Values are illustrated for y_{in} of the Reference Sample 1, y_{ir} of the reference median, and subsequent calculations of $|y_{i1} - y_{ir}|$, $(y_{i1} + y_{ir})$, and B-C. Example is modified from Environment Canada (2002).

	Taxon 1	Taxon 2	Taxon 3	Taxon 4	Taxon 5
Ref 1 (y_{i1})	2	3	2	3	1
Reference median (y_{ir})	4	4	2	3	1
$|y_{i1} - y_{ir}|$ or Ref 1 − reference median	2	1	0	0	0
$(y_{i1} + y_{ir})$	6	7	4	6	2

Substituting into Equation 36.4:

$$B\text{-}C = \frac{2+1+0+0+0}{6+7+4+6+2} = \frac{3}{25} = 0.12$$

where B-C is the dissimilarity value between the taxonomic composition of reference sample 1 and the median composition for the reference site.

TABLE 36.5	Example Calculation of the B-C Distance from the Reference Median to Each Reference or Exposure Sample. Example modified from Environment Canada (2002).

Reference or Exposure Site Sample Number	$|y_{i1} - y_{ir}|$	$(y_{i1} + y_{ir})$	Sample B-C distance to median	Mean($\pm$SE) B-C distance to median
Reference 1	3	25	0.12	
Reference 2	5	31	0.16	
Reference 3	11	27	0.41	0.18 ± 0.06
Reference 4	4	32	0.13	
Reference 5	2	30	0.07	
Exposure 1	26	54	0.48	
Exposure 2	17	41	0.41	
Exposure 3	17	43	0.40	0.43 ± 0.03
Exposure 4	23	45	0.51	
Exposure 5	13	39	0.33	

taxon I becomes y_{ir} in Equation 36.4. To determine whether there is an effect at the exposure site, the mean B-C distance between the reference stations and the reference median (0.18 ± 0.06 in Table 36.5) is compared to the mean distance between the exposure stations and the reference median (0.43 ± 0.03 in Table 36.5).

5. Compute the mean and standard error (SE) for each of the four endpoints above. Determine if there is a statistical difference between the reference and exposure sites for each of the four endpoints using a t-test to compare the two sample means (see Chapter 35 for a detailed statistical procedure). Record the mean (±1SE) values of the four endpoints (total abundance, richness, evenness, B-C index) for the reference and exposure sites in a data table.

B. Advanced Method 1: Determining Nutrient Limitation Using NDS Bioassays

Effluent discharges often contain nutrients that can stimulate food web productivity and contaminants that can lead to various levels of toxicity. This exercise employs NDS bioassays to determine whether the effluent discharge modifies nutrient limitation in the receiving waters. The simple and inexpensive technique follows the method outlined in Chapter 10 (also see Tank and Dodds 2003). Nutrient-diffusing substrates should be placed in the stream in uniform conditions of depth, current velocity and light conditions. NDS units are retrieved after a 21-d incubation period in the stream and processed for chlorophyll *a*.

1. Build the NDS following Basic Method 1 described in Chapter 10 and prepare 5 replicates of each treatment (i.e., control, N, P, N + P; 5 replicates × 4 treatments × 2 sites = 40 containers). Prepare data sheets as in Chapter 10 (Table 10.2).
2. Place the NDS in the field according to the Basic Method 1 of Chapter 10. Repeat the placement procedure until all four replicates are deployed at the reference and exposure sites.
3. Retrieve the NDS after 21 d and remove the disk from each container (see Basic Method 1, Chapter 10). Place the disk in a labeled plastic bag and store the sample on ice until frozen (within 4 h). Chlorophyll *a* should be processed following the protocol in Chapter 17.
4. Use a two-factor ANOVA (factors of N and P levels) to test whether algal biofilms were significantly affected by the treatments. Possible interpretations of the responses to nutrient treatments are provided in Table 10.1.

C. Advanced Method 2: *In Situ* Determination of Sublethal Effects of the Effluent

The bioassay described below uses the freshwater cladoceran, *Daphnia magna*, obtained from laboratory cultures. Using *Daphnia* offers key advantages. First, they are simple to culture in the laboratory (or may be purchased from biological supply companies), and, thus, do not deplete field populations. Second, and perhaps most important, their biology and responses to a wide range of toxic substances is well documented (USEPA 2005).

   This bioassay assumes that the user has access to *D. magna* cultures, and that food (algae) is readily available. Information on the culturing of *D. magna* is available from a wide range of literature sources, and is not covered further here. The *Daphnia* postexposure bioassay employs two key endpoints: mortality and feeding rate. Previous studies have demonstrated that, although feeding is generally more sensitive than mortality, the integrated response of these two endpoints allows evaluation of a wide range of scenarios from gross pollution to less obviously polluted sites (McWilliam and Baird 2002b, Slijkerman *et al.* 2004). The experimental design described below requires five replicate bioassay cages at the reference and exposure sites.

1. *Daphnia magna* can be cultured in a variety of media from filtered pond or well water to fully synthetic waters [e.g., hard water as described in ASTM (1995)]. Similarly, *D. magna* can be fed a variety of algal species as a diet, although the species most commonly used are green algae, usually *Chlorella vulgaris*, as it is a spherical cell that is simple to count using a variety of techniques. Fifty *D. magna* are required per field site (i.e., 100 animals in this example with a reference and

exposure site). Pour the culture medium into a 5-L plastic tank at a concentration of 6 mL/L. Add the green alga, *C. vulgaris*, at a concentration of 5×10^5 cells/ml. Add the required number of *D. magna* neonates (i.e., <24 h old). Maintain the animals at 20°C at a photoperiod of 16 h light:8 h dark. Add *C. vulgaris* to make a concentration of 5×10^5 cells/mL each day until the *D. magna* are 4 d old.

2. Once the *D. magna* are 4 d old, use a 3 mL pipette to collect the *D. magna* and randomly place them in groups of 10 in 175 mL screw-topped glass holding jars. (You will need 10 groups of 10 animals: 5 batches of 10 animals each for the replicate bioassay chambers at the reference and exposure sites.) Once all the animals have been allocated (i.e., 10 jars each with 10 animals), fill each jar completely with water and screw the lid on tight, ready for transport to the field. Next, fill sixteen 60 mL glass jars to the brim with 60 mL of *D. magna* medium containing *C. vulgaris* at a concentration of 5×10^5 cells/mL. These 16 jars with *C. vulgaris* remain in the laboratory as they will be used for the post-exposure feeding bioassay. At least five replicate bioassay jars are used for measuring post-exposure feeding rates and three additional bioassay jars are employed as blanks, and will contain no animals. Label the bioassay jars and transport to field.

3. At the field site, tie a tubular cage made of 4 mm wire or plastic mesh to a brick using nylon cord and place into the river. This mesh cage will serve to hold the exposure chambers in the river. Five plastic PVC exposure chambers with mesh windows are used for each site (Figure 36.1). To place the *D. magna* in the chambers, open and hold the chamber upright with the side with the remaining cap towards the bottom. Submerge it in the river, and pour the contents of the jar into the chamber and replace the cap quickly, placing each chamber into the cage. Once all 5 chambers have been placed in the cage, move the cage perpendicular to the water flow (Figure 36.1). Animals are exposed *in situ* for a 24-h period.

4. After 24 h, retrieve the mesh cages containing the exposure chambers. Under the water, remove each chamber from the cage and place into the 350 mL beaker, then lift out of the water. The beaker should be full of river water with the chamber resting in it. Remove the uppermost endcap from the chamber. With one hand holding the open end of the chamber and the beaker upright, place the neck of an empty 175 mL holding jar with the same diameter as the cages exactly over the chamber opening. Tip the contents of the chamber into the jar. Check the chamber to make sure no animals remain. Remove any remaining animals using a 3 mL pipette. Immediately transfer the containers with *D. magna* to the laboratory.

5. Record the number of animals recovered from the chambers (dead and alive), and any subsequent mortality among those recovered. Mortality differences among sites can be compared by converting the numbers of dead per chamber to a proportion, and taking the square root of the arcsin of the proportion. The transformed mortality values between sites can then be compared using a *t*-test as above. If significant differences in mortality exist between reference and impacted sites, there is no need to carry out the feeding analysis, as a gross effect has already been demonstrated. If there is no mortality during the exposure period, as is normally the case unless the site is grossly polluted, then the feeding rates of exposed animals can be assessed as outlined below.

6. Transfer 5 *D. magna* from each holding jar into a 60 mL bioassay jar with food. Put the bioassay jars into a cooler and close the lid (note that the temperature must remain close to ambient river values). Leave animals to feed postexposure for 4 h in the dark at the site of exposure. Animals cannot be transported while feeding. Remove
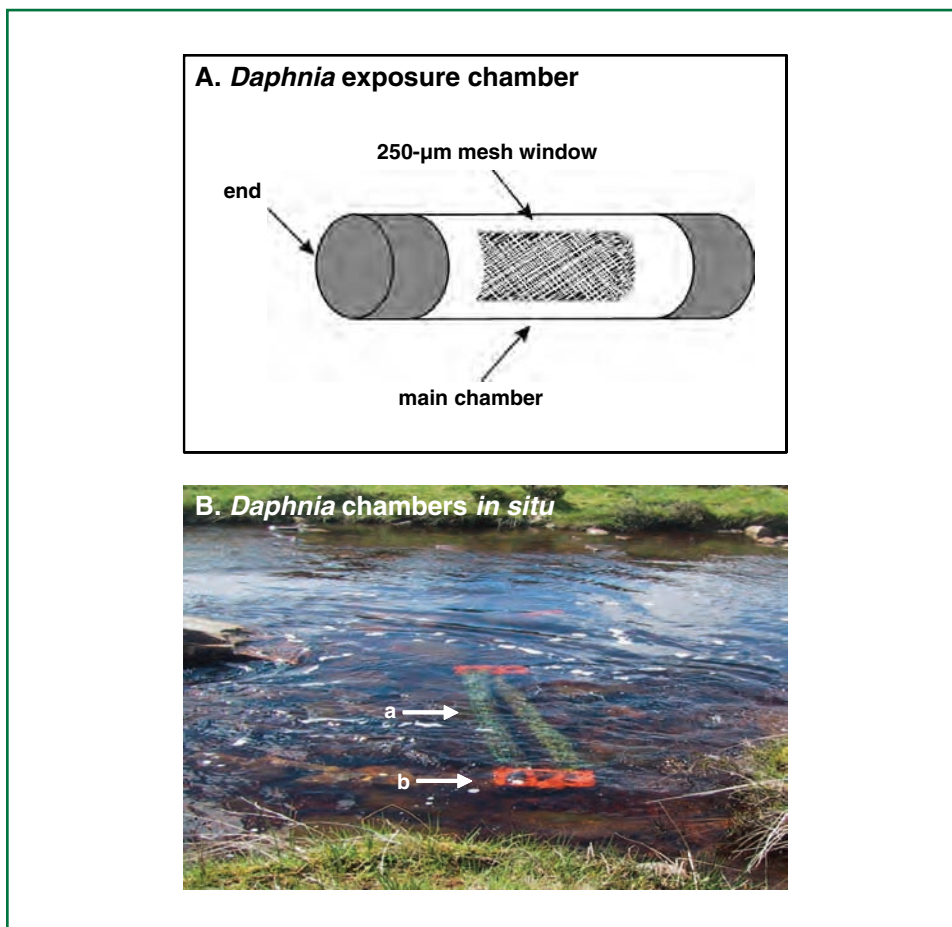
FIGURE 36.1 (A) Diagram of the *in situ* exposure chamber for the *Daphnia* bioassay; and (B) photograph of the bioassay chambers deployed in the (a) wire mesh cages that each contain five exposure chambers and (b) the anchoring brick.

animals from jars using a pipette after 4 h. Once animals are removed, the bioassay jars may be stored in a refrigerator at 4°C overnight, or frozen if necessary (*C. vulgaris* cells remain intact after freezing) before measuring feeding rates.

7. Feeding rate during the bioassay is measured using an indirect method, which estimates the amount of food eaten based on the number of cells remaining in the bioassay jar. The simplest method of assessing food remaining is to count the cells using an electronic particle counter, such as a Coulter Multisizer (Coulter Electronics, UK). If such an instrument is not available, then direct counts can be made using a compound microscope and a haemocytometer (see Chapter 16). Cell densities can also be estimated from the color of the cell suspension, using a spectrophotometer, although in this case, it is necessary to have previously derived a nomograph relating cell density to absorbance. For the spectrophotometric method, shake the bioassay jar containing the algal suspension and fill a 4 cm path-length cuvette. Measure the optical density of sample in a UV

spectrophotometer at 440 nm using bioassay medium without algae as a blank. Measure two samples for each jar. Convert the absorbance values obtained into cell density using the previously obtained nomograph.

8. Feeding rates are calculated using the equation given in Allen *et al.* (1995):

$$F = \frac{V \times (C_0 - C_t)}{t \times N} \tag{36.5}$$

where F is the feeding rate of single animal (cells/individual/h), V is the volume of suspension (mL), C_0 is the initial cell concentration (cells/mL), C_t is the final cell concentration (cells/mL), t is the time animals were allowed to feed (h), and N is the number of animals per replicate.

9. Determine if there is a statistically significant difference between the feeding rate of animals placed at the impacted site and that of animals placed at the reference site. This can be achieved by conducting a one-sided t-test comparing sites upstream and downstream of the effluent (H_0: feeding rate upstream $\leq$ feeding rate downstream H_A: feeding rate upstream $>$ feeding rate downstream). Alternatively, multiple sites can be compared using an ANOVA design, with differences between individual "impacted" sites and reference sites tested using the post hoc Bonferroni test (Zar 1999).

10. The only environmental factor that has been demonstrated to interfere with feeding following exposure in *D. magna* is the combination of high flow and inorganic suspended solids. It is important to note the presence (and, ideally, the nature and quantity) of any solids that are deposited in the exposure chamber during the bioassay, and to ascertain that flow rates have remained relatively constant over the deployment period.

D. Advanced Method 3: Separating Nutrient and Contaminant Effects on Benthic Food Webs

Experimentation using artificial streams complements biomonitoring and *in situ* bioassay studies because artificial streams provide control over relevant environmental variables and allow for the separation of multiple stressors contained within complex effluents (Lamberti and Steinman 1993, Culp *et al.* 2000b). In this example, we describe a general method that can assign cause-and-effect definitively, and that can isolate and measure the contrasting effects of nutrient and contaminant effects of effluents on benthic food webs. A detailed description of artificial stream design is beyond the scope of this chapter, and the reader is directed to the vast literature on this topic (Shriner and Gregory 1984, Kosinski 1989, Lamberti and Steinman 1993, Pontasch 1995, Rodgers *et al.* 1996, Culp *et al.* 2000d). The response variables (i.e., endpoints) are periphyton chlorophyll *a* and several benthic invertebrate measures (abundance, family richness, evenness, Bray-Curtis index) as described above.

1. Choose an appropriate artificial stream design. The simplest systems use flowthrough troughs located alongside the stream to which reference water is continuously delivered from a head tank. The head tank can be filled by pumps

or through piping that siphons water from upstream. Nutrient and effluent concentrations can be delivered to these simple systems using Mariotte bottles (see Chapter 8 for details of construction and operation). Although more complex techniques that use a series of delivery pumps have the important advantage of producing very precise nutrient and effluent concentrations (Figure 36.2; also see Culp *et al.* 2003), these techniques can be labor-intensive and costly.

2. The basic experimental design follows Culp *et al.* (2000c; 2003) and includes three treatments with at least four replicates: raw reference water collected upstream of effluent discharge; an N+P treatment simulating the nutrient concentration at the exposure site; and an effluent treatment simulating the exposure site concentration of effluent. Generally, the exposure concentration for the effluent and nutrient treatments is targeted to simulate the dilution ratio that corresponds to the stream reach where the effluent becomes completely mixed.

3. Effluent for the experiments should be collected from the effluent treatment system just prior to discharge to the river. Strictly follow all necessary safety precautions when handling effluent or other chemical solutions. N and P estimates for the full-strength effluent and the dilution ratio determined previously should be used to determine the N and P concentrations in the treatment additions.

4. Create a standardized benthic environment by adding washed gravel and cobble (from a nonimpacted site) to the bottom of each artificial stream to simulate typical riffle areas of the reference and exposure sites. The use of surface cobble from the
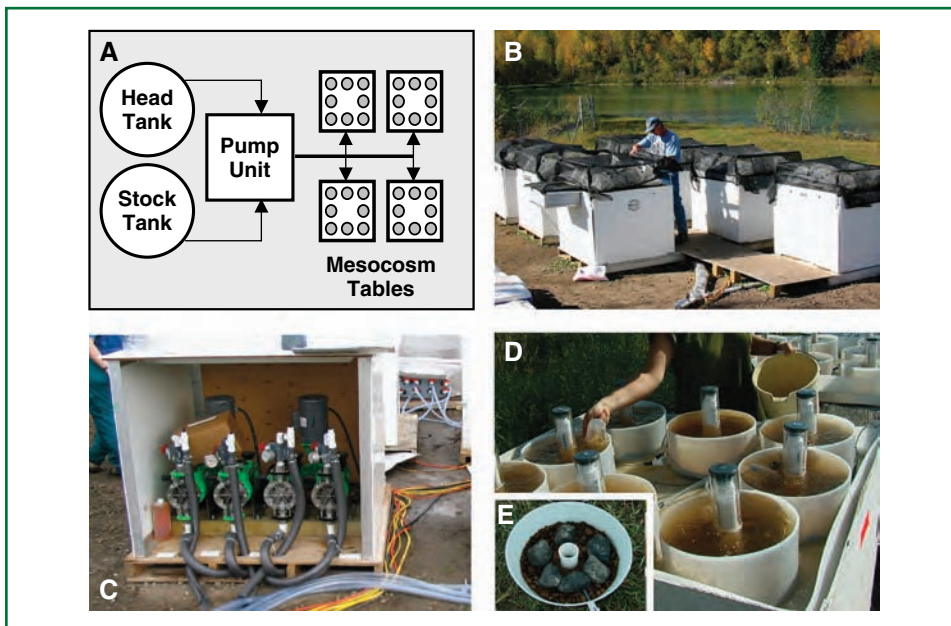
FIGURE 36.2 Mesocosm system used by Culp *et al.* (2003). (A) Schematic overview of mesocosm system with arrows indicating the direction of water flow; (B) photograph of mesocosm system deployed along the Wapati River, AB, Canada; (C) pumping unit containing four positive displacement, reciprocating pumps (Pulsa Feeder® Series Model 25 H), which transfer water from source tanks to the mesocosm tables; (D) mesocosm table containing eight artificial streams; and (E) an artifical stream with gravel and cobble substrate prior to experiment initiation.

reference site provides a method of stocking the streams with a natural periphyton community. Collect benthic invertebrates from the reference site using a Surber or Hess sampler. To prevent damage to the invertebrates, you must carefully lift the surface cobble and remove any attached invertebrates. Gently disturb the underlying substrate by hand to a depth of 5 cm. Carefully wash the collected invertebrates into a 1 L container filled with reference water, cover the container, and immediately transfer the invertebrates to the artificial streams. The number of benthic samples added to each artificial stream will depend on the total area of the artificial stream (e.g., 1 Surber sample per $0.1\,m^2$ of artificial stream channel). Note that benthic samples should be randomly assigned to stream channels. Allow reference water to flow through the systems for 1 d prior to beginning the nutrient and effluent additions. Monitor the artificial stream system each day to ensure that water delivery and flow rates are maintained, and that nutrient and effluent additions are continuously delivered.

5. Measure conductivity and collect water samples from each stream channel at weekly intervals. Analyze these water samples for nutrient concentrations.
6. After 21 d, collect samples of periphyton and benthic invertebrates from the artificial streams. Process these samples as described above. Use one-way ANOVA to determine if the treatments significantly affected algal or benthic invertebrate endpoints.

IV. QUESTIONS

1. Why do channel geometry, current velocity, and discharge need to be standardized between the reference and exposure site? Are there other physico-chemical variables that should be similar at these sites? Why?
2. How might a historical macroinvertebrate database aid you in selecting appropriate reference and exposure sites? If historical data are available, determine whether the effluent quality and quantity has changed substantially since the earlier macroinvertebrate survey. Were the collection methods used in the previous survey acceptable for a quantitative analysis? (Consider factors including mesh size of the sampling net, laboratory quality assurance and control, replication, etc.)
3. Where is the zone of complete effluent mixing at the exposure site? What is the disadvantage of not undertaking a conductivity survey? What assumptions are made when you estimate effluent concentration in the river based on simply calculating the dilution ratio from the ratio of effluent to river discharge volume?
4. Which chemical compounds in the effluent are most likely to affect the biota at the exposure site? Do any of the chemical concentrations exceed water quality guidelines?
5. Which benthic invertebrate response variables are expected to be sensitive to water pollution? What can you can you conclude about the effect of the effluent on the stream biota? For example, did the effluent affect productivity or biodiversity?
6. Is algal biomass limited by nutrient availability at the reference site? Was nutrient limitation relaxed by the effluent discharge?
7. Was Daphnia mortality or feeding rate significantly affected by exposure to the effluent? Was there any indication that the animals were exposed to high levels of suspended solids during the field experiment?
8. Contrast the results of the feeding and NDS bioassays. Do the field bioassays improve your ability to interpret the field survey results?

9. What are the advantages and disadvantages of employing artificial stream designs in retrospective risk assessment? What are the limitations of restricting the experiment to a 21-d period? How might data on insect emergence from the artificial streams help with the interpretation of effluent effects?
10. Use the weight-of-evidence approach outlined by Lowell *et al.* (2000) to integrate the results of the field survey, field bioassays, and artificial stream experiments. Using the results of this weight-of-evidence assessment, determine the major effects of the effluent on the benthic food web. What is the most plausible mechanism that explains the changes observed in the exposure site communities?

V. MATERIALS AND SUPPLIES

Basic Method

Site characterization

> Equipment for measuring channel geometry and substrate size (see Chapters 2 and 4)
> Equipment for measuring current velocity and discharge (see Chapter 3)
> Conductivity meter
> 1-L polyethylene bottles for collection of effluent

Field assessment of ecological effects

> Equipment for collection of water samples (see Chapters 9 and 10)
> Equipment for collection of benthic invertebrate samples (see Chapters 20 and 35)
> Equipment for collection of algal samples (see Chapter 17)
> Scintillation vials or bags for storage of algal samples

Laboratory methods

> Equipment for processing benthic invertebrate samples (see Chapters 20 and 35)
> Equipment for processing algal samples (see Chapter 17)

Advanced Method 1

> 32×60 mL plastic containers (centrifuge tubes are ideal)
> Agar
> KNO_3
> NaH_2PO_4
> Glass fiber filters
> 8 plastic test tube racks
> Stakes and nylon cord for attaching racks to streambed

Advanced Method 2

> *Daphnia magna* from laboratory cultures or a commercial source
> Algae from laboratory culture or a commercial source
> Culture medium
> 5-L plastic tank
> 10 PVC capped cages
> 10×175 mL screw-capped glass jars
> 5×13 mm^2 wire or plastic mesh cages
> 2 bricks

Nylon cord
350-mL Beaker
3-mL plastic pipettes
16×60 mL screw capped glass jars
Cooler
Coulter multisizer, a compound microscope and a haemocytometer, or a spectrophotometer for determining algal counts

Advanced Method 3

Artificial stream system (flow-through troughs are the simplest systems)
KNO_3 and NaH_2PO_4 for simulating effluent nutrient concentrations
Mariotte bottles (see Chapter 8 for details)
5-L plastic tanks for effluent transport (amounts will vary depending on stream design)
Washed gravel and cobble for artificial streams (quantities will vary depending on stream design)
Conductivity meter
Equipment for collection of water samples (see Chapters 9 and 10)

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