7 Welfare and 'best practice' in field studies of wildlife

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Introduction

Wildlife research is exciting and is an appealing career for many aspiring scientists. The life of the wildlife biologist or vet and the thrill of capturing and handling wild animals are glamorised by the media, and the professions are often treated as being synonymous with working to promote animal welfare and conservation. However, field studies of wild animals carry with them multiple risks for the animal subjects. Unfortunately, these risks and problems are sometimes given scant consideration by practitioners, often because they are judged relative to natural processes or are incurred 'for the good of the species' or population. Whilst wild animals undoubtedly suffer a range of markedly inhumane fates in the wild, and while some are in grave need of intervention for conservation and management reasons, the ethical/moral absolute of the welfare of the individual animal and the corresponding deep concern felt by society, mean that field research on wild animals requires an approach to ethical issues and the implementation of the Three Rs (3 Rs, see Chapter 2) that is as rigorous as for other areas of research using animals.

For a range of historical and subjective reasons, 'wildlife' is most commonly construed as naturally free-living vertebrates, most commonly mammals and birds, and to a slightly lesser extent, reptiles, amphibians and 'fish'. The huge diversity within and among these taxa, means that there is little consistency in their physiology, let alone their behaviour. Even within species, individuals and populations are likely to be behaviourally distinct; indeed this fine-scale variation is itself often the focus of field investigation. Generalising the needs and responses of individuals of particular populations and species is, therefore, an ambitious and probably unrealistic endeavour. All investigators and regulators must view field studies on a case-by-case basis, bringing relevant experience to bear where possible, but being prepared for exception and novelty. Since much of the legislation dealing with scientific procedures on animals is restricted to vertebrates (see Chapter 8), invertebrates will not be considered in this chapter. *Octopus vulgaris*, for example, is covered by legislation in the UK and, at a European level, protection may be extended to all cephalopods and decapod crustaceans. Kept animals, such as companion animals and farm livestock, will also not be considered. However, cats (*Felis catus*), dogs (*Canis familiaris*), pigs/boar (*Sus scrofa*), goats (*Capra aegagrus hircus*), horses (*Equus caballus*) and other kept animals, such as ferrets (*Mustela furo*), mink (*Neovison vison*), often return to a free-living state, sometimes outside of their native range, where they can revert to wild-type appearance and behaviour. Such feral animals are, to all intents and purposes, wild and so they fall within the scope of this chapter.

The object of field studies is to examine how wild animals behave in the wild or in as natural a situation and habitat as possible. Observations in the field might require little direct intervention, and be as apparently straightforward as observing animals from a distance, comparable to a birdwatcher's hobby. However, other treatments, procedures and initial observations might take place in the laboratory, or in other situations where wild animals are held captive for periods of time. Interventions might carry forward into the field, obviously when animals are equipped with tags or telemetry devices and less conspicuously if they are treated with internal markers, drugs or if their social or physical environment is manipulated around them. Therefore, this review extends to any interaction with wild animals in the field, the taking of animals from the wild and includes making observations or applying treatments in captivity, where captivity is temporary and takes place in the field or requires holding animals for periods of between a few hours and a few days. We will not dwell on the use of wild-caught animals in prolonged or terminal laboratory work, but focus on cases where animals are subsequently released back to the wild.

When working with animals in the wild, the work and its potential effects on the animals are often subject to uncertainty and unpredictability. It is never certain, for example, how many animals might be caught in a trap round or cannon net or how individuals might behave or respond to capture or interventions. With experience, many of these risks can be identified and mitigated. Expecting the unexpected and building appropriate contingency plans and budgets carries financial costs, but these are usually minor relative to the cost of mistakes and misjudgement which may lead to the projects being abandoned because of failures to comply with legislation and/or public opposition.

Reasons for wildlife research

The management of wild animals is an intrinsic part of land management. Often these practices have developed over centuries of common practice and form part of routine pest management for disease control and crop protection, as well as for food and sport. More recently, as awareness of the threats to biodiversity conservation has increased, management is frequently undertaken to enhance the status of threatened species. As society and the environment change, it is becoming much more important that we understand how wild animals might respond to management actions. Regulatory authorities and private sector interests can also intervene in management, by requiring evidence of humane treatment, efficacy or cost-effectiveness.

Studies may be carried out to examine wild animal biology, including population dynamics and individual behaviour and welfare. Wild animals are also used as indicators of environmental health. For these studies, the benefits in terms of knowledge, understanding and the conservation of the species are usually clearly articulated, however, the costs incurred to the individual animals are often not as well understood.

One critical difference between wild animal studies and the use of laboratory animals in research is that the subject of interest and focus of investigation is usually the subject animal in its own right rather than it serving as a model for the human condition or as a model of any other living system. For this reason, total replacement of animals in wildlife research (unlike, for example, clinical studies) will never be a feasible option (see Cuthill (2007) and Barnard (2007) for fuller discussions on this topic) and for many wildlife studies captive or captive-bred animals will never be a substitute for free-living animals. Nonetheless, due consideration should always be given to seeking alternatives where possible.

Welfare implications of wildlife studies

Effects of stress

All interactions between humans and animals have the potential to cause stress and behavioural or physiological changes and this is particularly likely with wild animals, where any kind of direct interaction is usually perceived as a threat. Stress is an integral part of all animals' lives and the body has developed many mechanisms for coping with both psychological and physical stressors (Broom & Johnson 1993). However, acute or prolonged stress can have diverse, profound and deleterious effects on the psychological and physiological health of animals and it is usually in the latter case that animals are said to be 'suffering from distress'. In wildlife studies the onset of these stress-related effects is of particular importance as the effects are difficult if not impossible to determine. This is because animals are normally released into the wild before any symptoms become apparent and, unlike laboratory subjects, wild animals are not constantly provided with the essentials of life (such as food, water, shelter) and so their survival is routinely challenged on a range of fronts.

In severe cases, acute stress can cause death from cardiac failure, but in the majority of instances the effects of acute stress will be more long term and harder to define. Physiological indicators of stress that may be observed during capture include shortness of breath (panting) and tachycardia (racing heart). However, from a behavioural point of view stress reactions are more difficult to ascertain and very much rely on having detailed knowledge of the biology of the study animals. For example, some animals become motionless (eg, rabbits (*Oryctolagus cuniculus*)) or may appear relaxed (eg, badgers (*Meles meles*)) when confronted with a stressor, whereas other animals will be hyperactive and spend a great deal of time in escape behaviours (eg, rats (*Rattus* spp.)). In addition, variation in response to stressors can occur within a species, with differences apparent in the reactions of dominant and subordinate animals and between genders (Overli *et al.* 2006).

The long-term physiological components of stress can affect most aspects of an animal's biology. High levels of stress hormones (such as glucocorticoids) are linked to poor reproductive success in males and females (Rivier & Vale 1984; Sapolsky *et al.* 2000) and reduced immunity from disease (Munck *et al.* 1984), both of which have the potential for significant impact on a wild animal's life and fitness.

Injuries

Apart from physiological effects there are also adverse physical consequences of field studies, primarily arising from capture and captivity. These range from minor injuries such as skin abrasions and tooth and claw damage in animals attempting to escape from cage or box traps and abrasions from external devices (eg, radiocollars) up to more severe injuries such as broken limbs (eg, birds in mist nets, 'foul' captures in traps), adverse reactions to drugs, predator attacks or death (eg, fish in gill nets).

One particularly serious problem associated with capture of wild animals is myopathy. This can occur when an animal is subjected to stress and intense physical exertion. It is unusual with cage trapping but can be found with netting or prolonged pursuit and handling of large mammals, particularly deer (Haulton *et al.* 2001). The condition is caused by a build up of lactic acid in muscles leading to stiffness, paralysis and, in extreme cases, eventual death (Conner *et al.* 1987). Symptoms normally have a delayed onset (sometimes over 1 week) and hence are rarely identified during capture. It is imperative, therefore, that stress levels are kept low by ensuring confident and effective handling and brief periods of pursuit and capture.

Other welfare effects

If the welfare status of an animal is unduly compromised this has repercussions, not only in ethical terms, but also on the validity and rigour of the scientific study itself. The stress associated with capture (and particularly when associated with anaesthesia) can have wide-ranging effects on an animal's biology, behaviour and ecology, and allowances must be made for these. Poor welfare arising from studies can have effects on the areas discussed in the following paragraphs.

Social structure and behaviour

The establishment and maintenance of social status within animal groups is complex and varies greatly with taxonomy, environment and among individuals. Social rank in a group is determined by many factors, but higher rank is often gained through aggressive behaviour linked to levels of the male hormone testosterone (Abbott *et al.* 2003; Schaffner & French 2004). Stress can alter levels of testosterone and hence may have an impact on the dominance hierarchies of a population, particularly in group-living animals such as wolves (*Canis Iupus*). In addition, even the temporary removal of an animal from its social group (especially the dominant male and female), may affect its position and the relative status of several others and be the cause of unrest and heightened aggression in the group. The effect of large-scale permanent removal of individuals on social structure has been well documented in badgers. Badger culling employed for the control of TB has resulted in disruption of territoriality, increased ranging behaviour and mixing between social groups (Carter *et al.* 2007).

Reproductive behaviour

The effect of stress on levels of sexual hormones is well documented and could have wide-ranging effects for males and females, with respect to their reproductive investment and success. Anaesthesia has the potential to cause abortion in the early part of gestation and premature birth in late pregnancy. It has also been shown that handling young can cause the mother to kill or abandon them. Therefore, where reproduction is not a focus of the research, studies might, where possible, avoid the sensitive parts of breeding seasons. There are also instances where the procedure itself can influence mating behaviour. This has been demonstrated in bird species that use 'badges of status', where ringing these birds can have an impact on mate choice (Burley 1986).

Foraging behaviour

Anaesthesia and stress can affect the metabolic condition and cognitive ability of an animal in the short term rendering it less able to forage. Alternatively, periods of captivity in the absence of preferred food may affect an animal's nutritional status and foraging choices upon release. This problem can be minimised if studies are conducted at times when there are fewer external pressures such as low food availability.

Spatial behaviour

Some animals have been shown to move away from an area from where they have experienced capture and anaesthesia presumably as the stress of this experience is associated with this geographical area (Teixeira *et al.* 2007). Others tend to be more sedentary than normal after capture and release. This has, potentially, wide-ranging effects on the population dynamics of group-living animals and also on the survival of individual animals that forsake their known home ranges and food sources.

While such changes may be temporary, resulting measures may give a misleading impression of typical ranging behaviour. Where observation periods are brief, these measures will be even more prone to bias.

Survival and mortality rates

Even the ringing of birds can have an effect on mortality in a population (Recher *et al.* 1985; Inglis *et al.* 1997). The procedure itself can directly affect the probability of survival and in addition, wild animals often carry underlying latent infections such as toxoplasmosis (eg, in sparrows) that may, as a result of lowered immunity due to stress, develop into clinical disease (Bermúdez *et al.* 2009).

Population dynamics

There is always the potential that the study itself might have a direct effect on the results. Increased mortality and disease are both possibilities but more subtle effects may be more difficult to determine. Moorhouse and Macdonald (2005) demonstrated that the sex ratio of a population of water voles was affected by the trapping and radio-tracking programme over a 3-year period. Over this time one discrete population of the water voles was regularly trapped and anaesthetised whereas a separate population was left undisturbed. After 3 years, both populations were trapped and the numbers estimated. It was found that the first population had a significantly higher ratio of males to females than the undisturbed voles. The conclusion being that the stress of capture and tracking had caused the mothers to produce higher levels of testosterone leading to an increase in male births.

Effects on others

One of the main differences in welfare terms between laboratory and wildlife studies is the fact that the latter have the potential to affect not only the study animals but also many other individuals in the surrounding area. Although this may not be avoidable it is always important to be aware of the consequences of any study and factor it into the ethical assessment.

Conspecifics

The effect of a study on an individual also has potential repercussions for conspecifics especially with respect to group living animals. This includes changes in dominance hierarchies and the onset of disease (see section on Survival and mortality rates).

Dependents

Removing parent animals from their dependents may cause malnutrition and, in severe cases, death of the young, particularly among animals with altricial young and those in the earliest stages of life. Treatment with drugs, including anaesthesia, has the potential to affect lactation (Yokoyama 1965), potentially exacerbating nutritional problems. Where breeding is not itself the focus of the study, trapping when young are dependent is a risk that should be avoided or mitigated. If there are obvious signs that the animals caught have dependent young (eg, lactation, brood patches), then it is advisable to release them as soon as possible, which may involve a judgement as to whether to carry out all of the intended procedures.

Non-targets
Capturing non-target species or individuals is almost unavoidable in wildlife studies, but the consequences can be more severe than those for the intended subject. For example, while the research might not be carried out during the breeding season of the subject it may be during that of non-target species; or the trap may not be appropriate (eg, weasels (*Mustela* spp.) caught in uncovered cage traps can die through hypothermia).

Three Rs and welfare

Exact numbers of wild animals used in regulated procedures are difficult to ascertain, and are not often collated specifically. In the UK, Home Office statistics[1] provide numbers of each species used in a range of subjects but it is not clear how many wild animals are studied in the field. For example 90 419 animals were used in the field of ecology in 2007. Of these 84 252 were fish, many of which were tagged and released as part of fisheries research though others were captive animals used in behavioural ecology research in the laboratory. The remaining individuals largely fell into the categories: Other rodent, Other carnivore, Other mammal (1405) and Other bird (3628) and Any amphibian (1027). Many of these studies of 'non-standard' species may have involved wild animals and will have been carried out at least partly in the field. In addition, wild species are also likely to have been used in other fields of study (eg, zoology, animal welfare). Many wildlife studies, including observational studies or minor interventions such as bird ringing, may not be regulated but nonetheless could affect the welfare of the subject animal.

Although the original definition of the Three Rs (replacement, reduction and refinement) was developed with laboratory studies in mind the principles and philosophy of this concept can be extended to many other areas in which there are human–animal interactions, as a means of ensuring the highest standards of welfare (Cuthill 2007). Unfortunately most of the information readily available with respect to the Three Rs tends to be aimed at their implementation in laboratory studies and many examples are not applicable to wildlife research (eg, cell culture, refinement of housing). This, however, should not lead to the conclusion that implementation of the Three Rs within wildlife research is not necessary or relevant. Here, a number of practical examples of how the Three Rs can be addressed in field studies is provided.

Replacement

Replacement is often not considered a viable option in studies of the behaviour and ecology of wildlife species, where the specific animals and their natural behaviour are intrinsic to the study (see section Reasons for wildlife research). However, there are alternative techniques that can give us a greater understanding of these topics without the use of animals themselves.

[1] http://www.homeoffice.gov.uk/rds

In silico studies (computer modelling)

This approach can be used to generate predictions of treatment effects, often taking into account uncertainty associated with observations and outcomes. In this way, the results of modelling can be more general than those of specific or localised observational studies. While such modelling requires understanding of the quality and representative nature of input data, modelling can also help evaluate the most important avenues of investigation allowing the field study to be refined. This approach has proven particularly effective in estimating population changes and for evaluating methods of management and disease control (Wilkinson *et al.* 2004).

Use of less sentient species

The substitution of a less sentient and/or non-protected species is usually classed as a form of replacement; this is not appropriate for the majority of wildlife studies, where the species itself is the subject of investigation. However, there is potential for using this type of replacement in ecotoxicology studies, where invertebrate models (eg, amphipods (*Gammarus pulex*)) can be used to assess levels of pollutants (Ashauer *et al.* 2007).

Read-across approach

This approach is more commonly associated with pharmaceutical and toxicity testing, usually in combination with computer modelling (Schultz *et al.* 2009). At a basic level it involves using data from one species to predict the outcome in others. Although this may not be appropriate for many field studies it may have uses, particularly in more heavily regulated areas such as ecotoxicology and developing population control methods. For example, if determining the effect of pesticides on non-target species data from wood mice (*Apodemus sylvaticus*) may be used to extrapolate to other small rodents such as harvest mice (*Micromys minutus*).

Reduction

Most of the principles and techniques of minimising animal use in the laboratory are also applicable to studies in the field.

Statistical design

The use of robust statistical approaches before, during and after the study can help ensure an efficient study where minimum numbers of animals are used and resources are deployed to best effect. Power analysis, where the project scale and sampling techniques are evaluated with respect to a range of probable effect sizes, is particularly important in planning investigations. It can now be applied to a range of complex analytical approaches, though this often requires repeated simulations of statistical outcomes rather than the off-the-peg power analyses available in standard packages (Dytham 2003).

As with laboratory studies, the precision and accuracy of observations in relation to the magnitude of any effect size are vital in considering the required sample size. Similarly variance in outcomes can be inflated by sampling across outwardly similar groups or environments, potentially masking treatment effects. Sampling design may need to be modified to account for, or avoid, these sources of potential variance. However, sample size and sampling design cannot be easily controlled in the field, and natural error variance and sampling error, if anything, tend to be more pronounced (Feinsinger 2001). The following factors often confound field investigations and should be taken into account in developing the study design:

- species, sex and age;
- weather conditions;
- presence and number of non-targets;
- interference by others (eg, members of the public).

The other factor to consider is that if the sample size is too small, repeating field studies to gain the data required is more difficult than in the laboratory, due to the inability to mimic the exact conditions used in previous trials.

Sequential testing

Sequential testing (or phasing) is where the sample size is not fixed in advance. Instead, data are evaluated as they are collected and further sampling is stopped in accordance with a predefined stopping rule as soon as significant results are observed. Thus a conclusion may sometimes be reached at a much earlier stage than would be possible with more classical hypothesis testing or estimation, with the potential to use fewer animals. With these techniques, as the study progresses, the design can be refined or the study halted as appropriate.

Using published or available data

Literature and other resources should be used to inform experimental design, perhaps in a power analysis, and hence reduce the number of animals or trials needed. It is important to note that the read-across approach works in this instance as well (ie, if no data are available for a particular species, searches should be made for data on related animals).

Sharing data

Data sharing is an important method for reducing animal use across the whole spectrum of animal-related studies. Data are normally shared within a scientific discipline via publications or presentations at conferences but this tends to focus only on positive results and finalised studies. It is as important, if not more so, that negative results and potential pitfalls of animal work are highlighted and a particularly good way of achieving this is by the use of specialist user groups on the web. These can provide an easily accessible and low-cost method to exchange data and ideas.

Samples can be shared as well as information. If different research teams (especially in the same geographical area) all require samples (eg, blood, hair, swabs) from the same species then it may be possible to work together so that the animals only need to be caught and sampled once. This needs to carry the caveat that the numbers, quantities and types of samples taken from an individual must remain within the best practice guidelines (see later in this chapter) and have the appropriate licensing authority.

Multi-use technique

This technique is particularly applicable to field use. It is a method of minimising the overall number of animals used by gaining data from one event that may be required for different parts of the study or for a completely separate study (eg, trapping animals for marking for ecological study and taking blood samples for a disease-monitoring programme). A good degree of forethought needs to be exercised when undertaking multi-use studies so that appropriate permissions are in place for all uses and any caveats observed before the studies are initiated.

Refinement

The main way of addressing refinement with respect to wildlife studies is through opting for the least invasive techniques and always to referring to 'best practice' guidelines and recent experience for capture, handling, marking and sampling appropriate for the species (see section Capture, handling, release). More specific, less-invasive methods are discussed in the following paragraphs.

Remote cameras

This method involves using remotely triggered cameras to gain information without any capture or manipulation of an animal. With digital technology, these cameras are more sophisticated and can create photographs in various formats, use time-lapse technology, can be motion-sensitive, and can operate at night using infra-red (Figure 7.1). Using video recording and/or still photographs has the advantage over direct observations in that there is less interference (and hence less stress imposed) on the animal and it requires less input from the investigator.

Non-invasive sampling methods

Many field studies involve taking samples to measure physiological parameters or pathological indicators. Although most of these factors have been traditionally measured in the blood, other less invasive approaches to sampling (eg, saliva and faeces) can be used in many instances to gain the same data. Faecal sampling is of particular interest as it can be used easily without disturbing the subject and without interfering with other welfare measures running in parallel, such as behavioural assessment (Lane 2006). Faecal sampling can be used to investigate hormone levels (eg, cortisol, testosterone), DNA analyses and for bait-marking purposes (Figure 7.2).

Identification

For some species natural markings can be used as a completely non-invasive method of identification (see Marking).

Figure 7.1 Image of wild boar using an infra-red camera trap.

Figure 7.2 Coloured beads as bait markers in badger latrine. A non-invasive and effective method to monitor bait uptake and to determine territory structure is to add coloured beads to baits which can then be detected in the faeces negating the need for any procedure or capture of the animal.

It is also important to be aware of techniques that should not be used in most circumstances due to the availability of more refined alternatives. A good example of this is the use of toe-clipping to permanently mark small mammals and amphibians. This invasive technique causes tissue damage (Golay & Durrer 1994; Reaser 1995), affects survival (Clarke 1972) and has been demonstrated to cause pain and suffering (May 2004). For these reasons it should, wherever possible, be replaced by a non-invasive technique such as natural markings or, if this is not possible, a less damaging technique such as microchipping.

With respect to refinement it is always important to consider the fate of non-targets as well as study animals. Non-targets may encounter the same potential costs to welfare as the target animals such as stress and injury caused by cage trapping or mist netting. Hence, it is vitally important that best practice methods of capture are employed to reduce the incidence of non-target captures (see next section).

Capture, handling, release

Most wildlife studies in the field include the use of capture, mark and release programmes. The techniques adopted in these programmes can have far-reaching consequences, so it is important to be aware of, and where possible, minimise the potential adverse effects not only on the study animals but also the other animals in the environment.

General welfare issues

Time of year

Research may lead to disruption of normal animal activities, whether as part of the study procedure or incidental to it. Disturbance of breeding individuals and dependent juveniles is of particular concern. Investigators should be aware of the breeding seasons of the species that they propose to study and ensure that there are no significant welfare implications associated with the timing of their research.

Time of day

An awareness of an animal's circadian activities is essential for appropriate capture and handling. Nocturnal animals should be kept in darkness when held in traps, as being away from cover during daylight hours will cause them further stress. Animals that are caught without the use of food baits (eg, mist netting of birds and bats) should be released with enough time to forage. If this is not possible, consideration should be given to provision of supplementary food and water or a glucose solution before release.

Extreme weather conditions (heat or cold)

Checking weather forecasts should always be a priority when carrying out field work. In the UK, the Met Office provide a pay-for service in which detailed, tailored forecasts can be sent directly to an email account. Trapping should be avoided during extreme weather conditions to reduce the possibility of hyper- or hypothermia. Shelter and extra warmth should be considered especially when anaesthetising animals in cold conditions (eg, heat pads, blankets, bubble wrap).

Non-targets

The capture of non-targets is always a possibility with live trapping so, where possible, use methods which maximise capture of the intended target and reduce capture of other species (eg, set restraining traps on a run). However, always be prepared to deal with non-targets if the need arises. In some cases certain non-target species, usually invasive non-native species (eg, grey squirrel, mink in the UK) may not be released back to the wild under conservation legislation, hence provision for humane euthanasia of these species should be made.

Capture

The choice of capture method should be made according to the species involved and availability of technology and personnel, potentially including veterinary cover. Methods of capture may be physical, such as the use of traps and nets. Small–medium mammals and birds are often caught in live traps, and netting tends to be the most common method for catching small birds and bats. Larger mammals may be trapped by a variety of methods (live trapping, netting) and may also be chemically sedated using darts. Capture efficiency and capture-related mortality rates have been found to differ considerably between methods and operators and this should be considered before a decision is made.

Live trapping

It is vitally important that only as many animals as can be effectively and safely dealt with in the time period available are caught. The number of cage traps set, should be the number able to be checked and processed within the allocated time (usually a maximum of 24h, though note that traps checked once in a 24h period could in theory hold animals captive for nearly 48h). It is also beneficial to have a good idea of variation in trap efficiency in the study system (eg, number of captures/100 trap night) so that it can be checked that the plan is achievable. Traps should always be securely shut down or wired open if they cannot be checked with the required frequency.

Being caught and held in a trap can be a very stressful experience for an animal but the trauma can be minimised by:

- avoiding exposed areas (so they are less likely to be bothered by predators, noise, etc);
- providing shade/cover and bedding or similar material where applicable – apart from calming the animal this can also reducing biting and scratching (Figures 7.3, 7.4);
- ensuring traps (and animals) are clearly labelled, particularly if the animals are being moved for processing so that it is known exactly where to return them for release;
- checking frequently – especially with small animals such as shrews (preferably at least twice a day);
- with netting, being able to close down the nets as the handling capacity is approached in the specified time period; small animals have high metabolic rates and can quickly lose condition, so efficiency is of the essence.

Most cage traps rely on a baiting system so food is usually available but it is also recommended that water or food with high water content is provided. In addition, if using live traps for small mammals, food for shrews (eg, fly pupae) should be provided even if they are not the target.

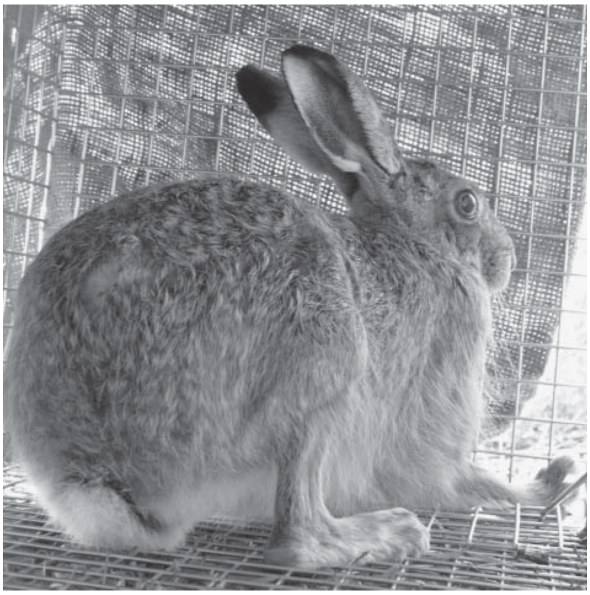

Figure 7.3 Brown hare (*Lepus europaeus*) in cage trap. Covering traps prevents exposure to the elements and predators and has a calming effect on most species.

Handling

Wild animals should not be handled unless necessary for the procedure. If handling is required the amount of contact should be kept to a minimum and the safety of the handler and of the animal needs to be considered (Figure 7.4). Wild animals are likely to bite or peck and scratch and are carriers of many zoonotic diseases (eg, *Leptospira*, *Cryptosporidium*) and so caution needs to be exercised at all times and risk assessments carried out before handling any wild animal. The method of handling will depend on the species of the animal and on the procedure to be carried out. However, there are key rules that should be followed:

- Handling should be kept to a minimum.
- Most animals like to be covered as it produces a calming effect. This is *not* the case for some species of deer (eg, muntjac (*Muntiacus* reevesi)), which find being covered more stressful.
- Handlers need to be confident and competent at dealing with the appropriate species.
- Rodents should never be lifted by the end of tail.
- With birds the hold must include the wings and legs in order to prevent damage to these appendages. Certain species may have specific requirements for physical restraint, including those with long legs and necks (Figure 7.5). Birds breathe by a bellows-like action of the ribs and sternum. Therefore, care should be taken so that the method of restraint does not interfere with the ventilatory movements of the sternum or impede the respiratory air flow.

Figure 7.4 Transferring wild rat from cage trap. When dealing with wild animals it is always beneficial if they can be studied with minimal handling. A simple black bag provides a device to extricate wild rats simply and safely from a cage trap. The rats, seeking cover in the darkness, will run directly into the bag avoiding direct handling.

Anaesthesia

Under Schedule 2(A) of Animal Scientific Procedures Act, anaesthesia should be used for all regulated procedures unless the use of these compounds is likely to cause more harm and distress. For wild animals, it may be that some procedures that are not inherently painful (eg, fitting a radiocollar) may still require sedation and/or anaesthesia, even if brief, for the handler's safety and the animal's welfare.

With all wild animal anaesthesia, veterinary input and advice should be sought from the outset. Doses, routes and recovery should be discussed fully with a veterinary surgeon before embarking on anaesthetising any wild animal. The following information is for guidance only.

After administering an anaesthetic the animal must be monitored to check that they are at the required depth of anaesthesia and that their vital body functions have not become dangerously depressed. Respiratory and cardiac function must both be monitored closely. When anaesthetising wild animals in the field, inhalation or injectable anaesthetics may be used (see Hall *et al.* 2000 for overview, and see also Flecknell 2009).

Inhalation anaesthetics

These types of anaesthetics tend to be used for small mammals and birds. There are portable versions of gaseous

Figure 7.5 Correct handling of a pheasant. Wings and feet are safely secured so the bird cannot damage itself or the handler.

Figure 7.6 Anaesthesia of a wild boar. Portable gaseous anaesthetic machines are an effective and safe way of maintaining anaesthesia (after initial darting or injection) in medium- and large-sized mammals in field situations.

anaesthetic machines (Figure 7.6) or anaesthetics can be delivered in a chamber in which the liquid compound is poured onto a gauze or cotton wool pad. The former set-up is preferable is it allows much more control of the depth and recovery from anaesthesia and hence should be used whenever it is possible and practical to do so. In the latter device the gas concentration is dependent on the temperature, and in cold winter weather it may be difficult to ensure that the anaesthetic agent actually vaporises. All volatile anaesthetics are irritant when in their liquid state, so the chamber must be designed to separate the anaesthetic-soaked gauze or cotton wool from the animal.

It can be more difficult to safely judge the correct depth of anaesthesia for birds. The avian respiratory system, which consists of a pair of relatively fixed lungs and a group of mobile air sacs, is more efficient at gas exchange than that of mammals and, therefore, birds will often demonstrate a more rapid response to the effects of inhaled anaesthetics. In addition, due to the large volume of stored gases in air sacs, birds can be slow to eliminate the anaesthetics. Recovery from anaesthesia can be facilitated by maintaining the bird in lateral recumbency and turning it every few minutes.

Injectable anaesthetics

These are commonly used for larger mammals. They may be delivered through a hypodermic syringe to a trapped or caged animal (eg, badgers, wild boar), or from a distance via a dart (eg, wild boar, deer) and will usually be delivered intramuscularly.

Injectable anaesthetics may be used to induce anaesthesia, which is then maintained with a gaseous anaesthetic. In some instances, particularly with small animals that are highly active, such as weasels (*Mustela nivalis*) the opposite may apply, with short-term anaesthesia induced by inhalation and maintained by injection. It should also be noted that the injectable agent ketamine (when used in isolation) has been shown to cause psychosis in humans particularly with repeated use and hence should be avoided for wild animal anaesthesia unless used in a cocktail with other drugs (such as metomidine) (de Leeuw *et al.* 2004).

Darting

Use of a dart-gun to dart wild animals is a specialist skill and may be subject to a number of restrictions. In the UK, dart rifles and blow pipes are classified as Section 5 prohibited weapons under the Firearms Act and require both a permit from the Home Office and permission from the statutory authority before use. Furthermore, there are restrictions on the number of darts that may be held by one individual. Details may vary between authorities and advice should always be sought. When firing a dart, the aim is for the dart to hit the animal at right-angles, ensuring that the needle penetrates the subcutaneous fat and enters the muscle. The propellant pressure of the rifle should be adjusted depending upon the distance between the operator and the animal to ensure that the dart has sufficient velocity to penetrate to the muscle, but not so much that it enters the body cavity. It is essential to practise thoroughly and regularly before using this equipment on animals.

Sampling

Methods and volumes for blood sampling of wild species are similar in many instances to those for laboratory species (Joint Working Group on Refinement (JWGR), 1993), although sedation or anaesthesia may be needed for restraint. Due consideration should be given to the health and physiological status of the animal as this can affect the level of handling or the amount of blood withdrawal that can be safely undertaken without causing the animal to go into hypovolaemic shock. As with laboratory animals, no more blood should be taken than is necessary and where alternative less invasive methods are available (eg, use of saliva (Figure 7.7) or faeces) these should be chosen.

If hair and/or whiskers (vibrissae) are to be taken, cutting/shaving rather than plucking is preferable if the follicles are not required. Whiskers should be taken equally from both sides of the face and only a small percentage of the total number of whiskers should be removed at any one time. Feathers should be taken from less essential areas of birds (eg, from the back rather than the wings). For stable isotope

Figure 7.7 Salivary sampling of a serotine bat. Saliva can be collected without anaesthesia in many species and used instead of blood for a variety of physiological measures (eg, immunoglobulins, stress and reproductive hormones).

sampling of tissues it is important to consider the time at which tissues were formed.

Risk of infection is high in wild animals (perhaps particularly in fossorial species) and hence the use of antibacterial sprays and/or use of surgical glue, is recommended for skin protection following invasive sampling.

Marking

Recognition of individual animals plays an important part in most wildlife research. Marking can provide information about survival, site fidelity, population dynamics, social behaviour, feeding ecology and almost every facet of an animal's ecology. Several techniques are available (see also Chapter 18), such as:

- telemetry: external and internal, VHF, GPS and proximity transmitters;
- external ringing and tagging (bird and bat banding, mammal ear tags, wing tags);
- physical marking (tattooing, fur clipping, scale marking);
- internal marking (microchips, fish wire tags);
- natural markings.

Telemetric/GPS devices

A wide variety of attachment methods for both types of transmitters exists (collars, tags, implants). External devices should be as light in weight as possible and should not usually exceed 5% of the body mass of the animal (<3% is recommended). Devices that break away after sampling, at the end of the useful life of the transmitter or those with a remote release are preferable. Collars/harnesses should always be fitted to allow room for growth and natural variation in body mass, which can be pronounced in some species. For example, when fitting collars on small–medium mammals, insert fingers between the neck and the collar to judge the appropriate fit (Figure 7.8).

Figure 7.8 Fitting a radio collar to a red fox. It is essential that fitting of the collar is comfortable and allows for growth.

Ringing

This is the most accepted method of marking birds. In the UK, ringing is not regulated under legislation relating to animals used in research but attachment of any marks or tags to wild birds requires formal training and a permit from the British Trust for Ornithology[2]. Scientists can obtain scientific permits that are more restricted in their scope, but may require less diverse training and accreditation.

Tagging

When tagging animals, bright colours should be used with caution as they may affect camouflage and act as an attractant for predators – they also make the animals visible to members of the public. In addition, tags should be thoughtfully placed so that they are not likely to snag or get caught on vegetation, potentially leading to tissue damage, this is of particular relevance for animals that squeeze through crevices or holes (eg, bats, rats).

Physical marking

Fur clipping is a good non-invasive method of temporary marking an individual but its use is limited in most studies due to its short duration. In contrast, tattooing is a permanent mark and of great benefit in long-term population studies. However it should be noted that it always requires anaesthesia and does pose a risk of infection (especially for fossorial animals) and hence the use of antiseptic sprays or creams on the tattooed area is recommended.

Microchipping

Microchipping or the use of passive integrated transponders (PIT tags) represents a relatively recent advance in animal marking technologies (Camper & Dixon 1988) and has become the most popular method of choice for many small mammals and amphibia. The tag itself is a small cylinder (approximately 12 mm in length) and it is usually injected

[2] http://www.bto.org/ringing

subcutaneously in the scruff of the neck or can be implanted in the lymphatic cavity (particularly in amphibians or reptiles). Although available data suggest no strong evidence for lasting detrimental effects of these tags (Brown 1997) most studies concentrate on efficacy and cost rather than welfare, behaviour, growth and survival. There is anecdotal evidence suggesting that subcutaneous PIT tags can occasionally migrate, potentially leading to problems with the tag moving around the scapular region or even being expelled from the body. There is also some concern that implanting tags into the abdomen through the muscle is a relatively invasive procedure and has the potential to cause pain, necrosis of tissue and/or inflammation around the site. In addition, in some cases PIT tags are not retained as reliably as other marking techniques (Ott & Scott, 1999).

Natural marking

Individual identification based upon natural markings is an under-utilised refinement method. The theory behind individual identification involves the use of physical markings, patterns or coloration to distinguish between conspecifics. The advantage of this method is that it enables identification without extended periods of handling, therefore minimising disturbance to the animal. It also has low cost compared to other methods (Doody 1995). The age of digital photography has also provided a method of storing a large number of pictures, which can be viewed easily and transferred between facilities. The method has now been used for range of different species including many types of amphibian (Doody 1995; Loafman, 1991), cetaceans (Rugh *et al.* 1992; Neumann *et al.* 2002), birds (Bretagnolle *et al.* 1994), cheetahs (Kelly 2001) and whalesharks (Arzoumanian *et al.* 2005).

Invasive tissue marking

Marking techniques that cause significant tissue injury, such as branding and toe, ear and tail clipping, should be avoided. If no alternative methods can achieve the desired results then researchers need to ensure that the marking process does not cause unnecessary tissue damage, pain, and/or severe blood loss. Adequate pain control is a necessity when undertaking such procedures.

The method used will depend on the species and type of study. When choosing a marking technique, primary consideration should be given to methodologies that are the least invasive, do not require recapture for identification, and will remain visible for the duration of the study. In addition, marks should:

- be quick and easy to apply;
- be readily visible and distinguishable;
- persist on animals until all research objectives are fulfilled;
- not introduce bias by having variable tag retention rates;
- not cause long-term adverse effects on health, behaviour, longevity or social life;
- comply with any legal restrictions or regulations;
- allow for seasonal changes in mass and growth of juvenile animals.

Release

Animals should be released back to the point of capture when fully recovered from the procedures performed. If an animal is injured or showing signs of illness euthanasia may be required. The most humane method of dispatch in the field will depend on the species and the experience of the investigator. Table 7.1 lists some suggested methods but see also Chapter 17 and AAZV (2006). The investigator should always have the necessary equipment to euthanase target or non-target animals where appropriate (eg, anaesthetics for overdose) or, in the case of larger animals where veterinary advice may need to be sought, contact numbers for 24-hour cover.

To treat or not to treat?

There are often ethical and moral dilemmas to be faced in the treatment of wild animals. Interference with a natural process could lead to perturbation of the ecological balance and this must be considered when deciding whether or not to treat a wild animal. As a general guideline, it is accepted that injuries and illnesses that have anthropogenic causes should be treated (see Figure 7.9 for an example flow chart). In many other cases, the researcher must make an informed choice based upon his/her knowledge of the animal, the injury (or illness) and the situation. The final decision should be one that the researcher has the ability and confidence to defend.

Legislation appropriate to wildlife studies

Anyone proposing to conduct research on, study, capture, hold or release wildlife should be familiar with, and comply

Table 7.1 Methods of dispatch. Please note that these methods are not the only, or necessarily the most appropriate methods to be used in all situations. The method of dispatch used should always be decided depending on the health status of the individual, the situation, the setting, the competence of the personnel and local regulations.

Animal	*Suggested method of dispatch*
Small rodents Rats Birds	Dislocation of neck or overdose of gaseous anaesthesia
Rabbit	Dislocation of neck (requires highly skilled operator)
Hedgehog	Overdose of gaseous anaesthesia
Badger	Overdose of anaesthesia (injectable)
Bats	Overdose of gaseous anaesthesia
Fox Deer Wild boar	Overdose of anaesthesia (injectable) or shooting

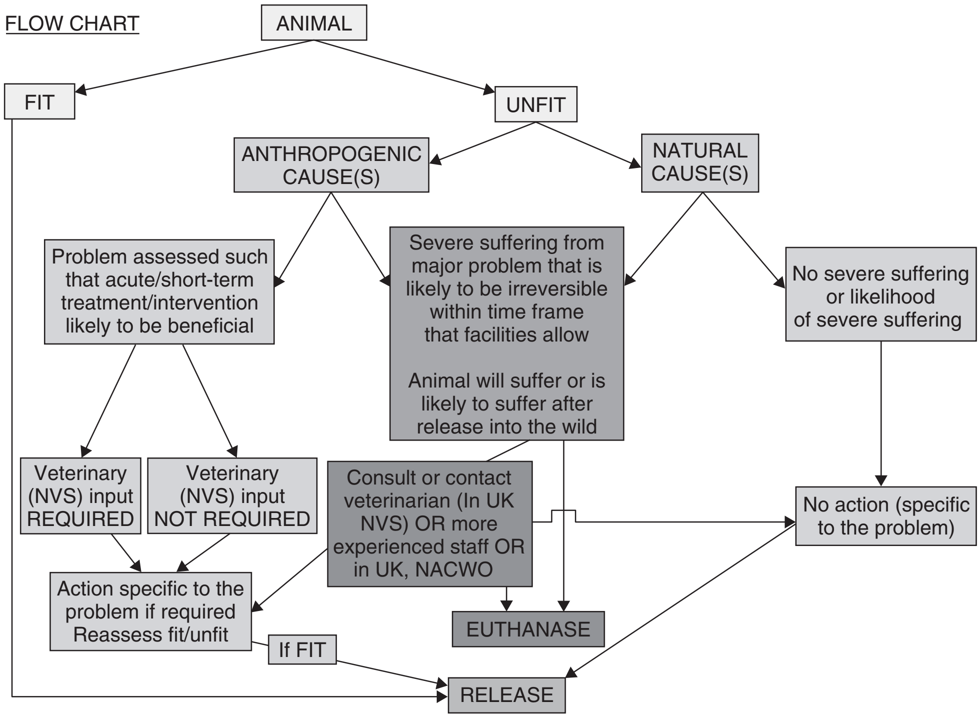

Figure 7.9 Example flow chart on dealing with injured animals in the field. It is vital that all field workers are aware of how to deal with injured target and non-target animals. This flow chart shows an example of strategies for dealing with injuries that are due to the procedure (iatrogenic) or for which the animal sustained prior to capture. In the UK veterinary advice is likely to come from the named veterinary surgeon (NVS). NACWO stands for named animal care and welfare officer, who in the UK is responsible for the day-to-day care of protected animals used in research.

with, the relevant legislation governing their use. In many cases, licences or permits are required to conduct work with wildlife. As an example, the paragraphs below list some of the provisions pertaining to wildlife research in the UK.

Legislation relating to the use of animals in research

In the UK, the Animals (Scientific procedures) Act 1986 (A(SP)A) states that all regulated work must be carried out a designated establishment (DE) unless the work requires:

- wild animals or farm species at sites that could not be reasonably part of a DE;
- studies that depend upon access to the wild environment or commercial husbandry standards.

When regulated work under A(SP)A is carried out at field sites these are classed as PODEs (places other than designated establishments). At PODEs procedures must be conducted and welfare standards maintained as near as practicable to those achievable in DEs. Additional conditions also apply to PODEs to enable appropriate controls to be applied, such as notification conditions (ie, usually the inspector is required to be notified of all PODE sites at least 72h before the onset of a regulated study). Land-owner's consent, where appropriate, must be obtained prior to applying for a licence under A(SP)A and provision must be made to allow inspectors onto all PODE sites.

Wildlife studies and/or techniques that are not regulated under A(SP)A include:

- the ringing, tagging or marking of an animal or the use of any other humane procedure for the sole purpose of enabling an animal to be identified is not a regulated procedure under A(SP)A if it causes only momentary pain or distress and no lasting harm;
- humane killing by a recognised method;
- procedures applied in the course of recognised veterinary practice;
- capture and release of wild animals unless the method of capture itself is being studied.

However, it should be noted that any of the above do become regulated if anaesthesia is used. Advice should always be sought from the local Home Office inspector in case of any doubt as to whether a procedure is regulated or not.

Certain species to be used in research may be obtained only from a designated breeding establishment, unless an official exemption is granted. Therefore, it is also illegal to trap these animals in the wild without an exemption granted by the Home Office. The animals on this list include mouse, rat, rabbit, ferret and quail. Moreover release of animals back to the wild will only be authorised if:

- the maximum possible care has been taken to safeguard the animal's well-being;
- the animal's state of health allows it to be set free;

- setting the animal free poses no danger to public health and the environment.

A(SP)A licences do not absolve the licensee from their duties under other wildlife legislation. Hence all work must comply with other appropriate legislation and other applicable licences must be in place before any studies commence.

Wildlife and Countryside Act

The Wildlife and Countryside Act 1981 is the principle mechanism for the legislative protection of wildlife in Great Britain but does not extend to Northern Ireland, the Channel Islands or the Isle of Man; the Wildlife (Northern Ireland) Order is equivalent in many respects. Most countries have similar legislation, particularly in the EU. This legislation is the means by which the Convention on the Conservation of European Wildlife and Natural Habitats (the 'Bern Convention') and latterly the Council Directive on the Conservation of Wild Birds (79/409/EEC) are implemented in Great Britain. Similar legislation is enacted to fulfil these obligations elsewhere in the United Kingdom. The Wildlife and Countryside Act (WCA) is divided into four parts with Part I being concerned with the protection of wildlife.

Part I of WCA protects all wild birds and protected animals (includes some mammals, all species of bat; species of dolphin; porpoise; otter; amphibians; reptiles; and many species of insects). A wild bird is defined as any bird of a species that is resident in or is a visitor to the European Territory of any member state in a wild state. Under the WCA it is an offence to:

- take, injure, kill or sell a protected species;
- disturb a protected species in its nest or place of shelter;
- possess a protected species.

There are additional clauses and various additional forms of protection.

However, many activities prohibited under the WCA can be carried out after acquiring a licence issued by the appropriate authority to avoid committing an offence. For example scientific study that requires capturing protected animals can be allowed by obtaining a licence.

Other wildlife legislation

The Conservation (Natural Habitats, etc) Regulations 1994

These implement the Council Directive 92/43/EEC in Great Britain under which it is an offence, with certain exceptions, to:

- deliberately capture or kill any wild animal of a European protected species;
- deliberately disturb any such animal;
- deliberately take or destroy eggs of any such wild animal;
- damage or destroy a breeding site or resting place of such a wild animal;
- deliberately pick, collect, cut, uproot or destroy a wild plant of a European protected species;
- keep, transport, sell or exchange, or offer for sale or exchange, any live or dead wild animal or plant of a European protected species, or any part of, or anything derived from such a wild animal or plant.

Wild Mammal Protection Act 1996

This act covers unprotected mammals to prevent unnecessary suffering by certain methods such as self-locking snares, explosives, drowning, asphyxiation and use of live decoys. The Wild Mammal Protection Act 1996 does not apply in legal pest control or in the humane killing of an injured animal.

Animal Welfare Act 2006

This act ensures that it is not only against the law to be cruel to an animal, but also the welfare needs of the animals must be met. A 'protected animal' under this act is domesticated, not living in a wild state, or under control of man (either permanently or temporarily). The latter does include wild animals captured even for a short period. An offence is caused when an '*act of a responsible person causes an animal to suffer … and suffering is unnecessary*'. '*Suffering for a legitimate purpose*', eg, research is permissible but only if suffering is proportionate to purpose of the conduct, and it could not have been avoided or reduced, and the conduct concerned was that of a reasonably competent and humane person.

CITES

The Convention on International Trade of Endangered Species provides protection to specified endangered species and on the taking, handling and transport of samples taken or collected from them[3]. This can constrain the international movement of samples collected for scientific purposes so advice should always be sought about the application of CITES regulations in research on CITES listed species.

Other examples of legislation include acts that are designed for the protection of specific groups of wildlife. In the UK this includes acts such as:

- The Protection of Badgers Act 1992;
- The Deer Act 1991;
- The Ground Game Act 1880;
- The Whaling Industry (Regulation) Act 1934;
- The Conservation of Seals Act 1970;
- The Salmon and Freshwater Fisheries Act 1975;
- The Dangerous Wild Animals Act 1976;

Some of these acts have parallel legislation in Scotland and Northern Ireland.

Licences

Natural England (the Countryside Council for Wales, Scottish Natural Heritage and Northern Ireland Environ-

[3] http://www.defra.gov.uk/animalhealth/CITES/

ment Agency have similar arrangements) is responsible for issuing licences and permits through their Wildlife Management and Licensing Service under a range of wildlife legislation for activities that would otherwise be illegal but where a valid justification exists.

International legislation

Many countries have their own guidance and legislation regarding the use of animals in research and the protection of animals in the wild. It is important that researchers are aware of the local regulations in different countries and abide by these and the best practice guidelines (see below). Links and information are available regarding animal research and welfare legislation for many areas in the world, including USA, New Zealand, Canada and Europe[4].

Best practice guidelines

The key to carrying out wildlife studies in the field to the highest standards is to follow best practice guidelines wherever possible. Below is a selection of relevant websites, most of which have links to further resources.

UK

National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs)

http://www.nc3rs.org.uk

Microsites on a number of topics with relevance to field studies; of particular interest is their site on Three Rs and wildlife research, but also there are sites on dosing and sampling and anaesthesia that include information appropriate to studying animals in the field.

Association for the Study of Animal Behaviour (ASAB)

http://asab.nottingham.ac.uk/ethics/guidelines.php

Guidelines relating to conducting animal research with some areas particularly associated with field studies (eg, marking) and also has links with other websites that contain information with respect to welfare and ethical treatment of animals.

British Association for Shooting and Conservation (BASC)

http://www.basc.org.uk/

Although not a welfare organisation, BASC has codes of practice on trapping of pest mammals and pest birds which include information on the different legislations, and practical tips on topics, such as how to reduce non-targets and the appropriate setting and positioning of traps which may be of help in conducting field studies of these species.

[4] http://www.animalethics.org.au/legislation/international

Worldwide

Norwegian Consensus-platform for Replacement, Reduction and Refinement of Animal Experiments (norecopa)

http://www.norecopa.no

Guidelines for wildlife research particularly relating to the Three Rs.

Canadian Council for Animal Care (CCAC)

http://www.ccac.ca

Three Rs microsite with a special section on its implementation on wildlife research and refinement alternatives for marking and tagging.

Concluding remarks

This chapter is intended as a signpost to the issues that a potential researcher should be considering but does not cover all outcomes that are possible in an ever-changing environment. Before embarking on a field study, preparation is key. A checklist of considerations can be of great benefit, an example of which is given below:

- Do you need to use animals to achieve your aims?
- Are you using the lowest number of animals to achieve your aims?
- Are you using the least invasive but effective methods?
- Have you checked best practice guidelines?
- Have you got the appropriate legal authorities (eg, licences)?
- Have you sought advice from others (eg, veterinary surgeon)?
- Have you checked the weather forecast?
- Have you checked breeding seasons?
- Have you checked your field equipment is the most appropriate for your target species and is fully functional?
- Have you minimised non-target risk?
- Do you know how to treat/dispatch injured animals?
- Do you know how to check and ensure the welfare of the animals before discharging them from your care?

The wildlife researcher has to be prepared for any eventuality and ensure the welfare of the animals within their care is maintained at the highest possible level. This is best achieved by always considering the Three Rs, being aware of best practice guidelines and taking advice from colleagues and other experts in the relevant scientific fields.

References

AAZV (2006) *Guidelines for Euthanasia of Nondomestic Animals*. American Association of Zoo Veterinarians. http://www.aazv.org (accessed 21 July 09)

Abbott, D.H., Keverne, E.B., Bercovitch, F.B. *et al*. (2003) Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. *Hormones and Behavior*, **43**, 67–82

Ashauer, R., Boxall, A.B. and Brown, C.D. (2007) New ecotoxicological model to simulate survival of aquatic invertebrates after exposure to fluctuations and sequential pulses of pesticides. *Environmental Science and Technology*, **41**, 1480–1486

Arzoumanian, Z., Holmberg, J. and Norman, B. (2005) An astronomical pattern matching algorithm for computer-aided identification of whalesharks Rhincondon type. *Journal of Applied Ecology*, **42**, 999–1011

Barnard, C. (2007) Ethical regulation and animal science: why animal behaviour is special. *Animal Behaviour*, **74**, 5–13

Bermúdez, R., Faílde, L.D., Losadab, A.P. *et al*. (2009) Toxoplasmosis in Bennett's wallabies (*Macropus rufogriseus*) in Spain. *Veterinary Parasitology*, **160**, 155–158

Bretagnolle, V., Thibault, J. and Dominici, J. (1994) Field identification of individual osprey using head marking pattern. *Journal of Wildlife Management*, **58**, 175–178

Broom, D.M. and Johnson, K. (1993) *Stress and Animal Welfare*. Blackwell Publishing, Oxford

Brown, L.J. (1997) An evaluation of some marking and trapping techniques currently used in the study of anuran population dynamics. *Journal of Herpetology*, **31**, 410–419

Burley, N. (1986) Sexual selection for aesthetic traits in species with biparental care. *American Naturalist*, **127**, 415–445

Camper, J.D. and Dixon, J.R. (1988) Evaluation of a microchip marking system for amphibians and reptiles. *Journal of Herpetology*, **22**, 425–433

Carter, S.P., Delahay, R.J., Smith, G.C. *et al*. (2007) Culling-induced social perturbation in Eurasian badger *Meles meles* and management of TB in cattle: an analysis of a critical problem in applied ecology. *Proceedings of The Royal Society of London B*, **274**, 2769–2777

Clarke, R.D. (1972) The effect of toe clipping on survival in fowlers toad (*Bufo woodhousei fowleri*). *Copeia*, **1**, 182–185

Conner, M.C., Soutiere, E.C. and Lancia, R.A. (1987) Drop-netting deer: costs and incidence of capture myopathy. *Wildlife Society Bulletin*, **15**, 434–438

Cuthill, I.C. (2007) Ethical regulation and animal science: why animal behaviour is not so special. *Animal Behaviour*, **74**, 15–22

Doody, J.S. (1995) A photographic mark-recapture method for patterned amphibians. *Herpetological Review*, **26**, 19–21

Dytham, C. (2003) *Choosing and Using Statistics. A Biologists Guide*. Blackwell Publishing, Oxford

Feinsinger, P. (2001) *Designing Field Studies for Biodiversity and Conservation*. Island Press, Washington, DC

Flecknell, P.A. (2009) *Laboratory Animal Anaesthesia*, 3rd edn. Academic Press, London

Golay, N. and Durrer, H. (1994). Inflamation due to toe clipping in natterjack toads (*Bufo calamita*). *Amphibia–Reptilia*, **15**, 81–83

Hall, L.W., Clarke, K.W. and Trim, C.M. (2000) Anaesthesia of birds, laboratory animals and wild animals. In: *Veterinary Anaesthesia*, 10th edn., pp. 463–478. Elsevier Press Ltd, London

Haulton, S.M., Porter, W.F. and Rudolph, B.A. (2001) Evaluating 4 methods to capture white-tailed deer. *Wildlife Society Bulletin*, **29**, 255–264

Inglis, I.R., Isaacson, A.J., Smith, G.C. *et al*. (1997) The effect on the woodpigeon (*Columba palumbus*) of the introduction of oilseed rape into Britain. *Agriculture Ecosystems & Environment*, **61**, 113–121

Joint Working Group on Refinement (1993) Removal of blood from laboratory mammals and birds. First Report of the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals*, **27**, 1–22

Kelly, M.J. (2001) Computer-aided photograph matching in studies using individual identification: and example from Serengeti cheetahs. *Journal of Mammalogy*, **82**, 440–449

Lane, J. (2006) Can non-invasive glucocorticoid measures be used as reliable indicators of stress in animals? *Animal Welfare*, **15**, 331–342

de Leeuw, A.N.S., Forrester, G.J. and Spyvee, P.D. (2004) Experimental comparison of ketamine with a combination of ketamine, butorphanol and medetomidine for general anaesthesia of the Eurasian badger (*Meles meles L.*). *Veterinary Journal*, **167**, 186–193

Loafman, R. (1991) Identifying individual spotted salamanders by spot pattern. *Herpetological Review*, **22**, 91–92

May, R. (2004) Ecology: ethics and amphibians. *Nature*, **431**, 403

Moorhouse, T.P. and Macdonald, D.W. (2005) Indirect negative impacts of radio-collaring: sex ratio variation in water voles. *Journal of Applied Ecology*, **42**, 91–98

Munck, A., Guyre, P.M. and Holbrook, N.J. (1984) Physioloigical functions of glucocrticoids in stress and their relation to pharmacological actions. *Endocrine Review*, **5**, 25–41

Neumann, D.R., Leitenberger, A. and Orams, M.B. (2002) Photo identification of short beaked common dolphins (*Delphinus delphis*) in north east New Zealand: a photo catalogue of recognisable individuals. *New Zealand Journal of Marine and Freshwater Research*, **36**, 593–604

Ott, J.A. and Scott, D.E. (1999) Effects of toe clipping and PIT-tagging on growth and survival in metamorphic Ambystomaopacum. *Journal of Herpetology*, **33**, 344–348

Overli, O., Sorensen, C. and Nilsson, G.E. (2006) Behavioral indicators of stress-coping style in rainbow trout: Do males and females react differently to novelty? *Physiology & Behavior*, **87**, 506–512

Reaser, J. (1995) Marking amphibians by toe-clipping; a response to Halliday. *FROGLOG*, **12**, 1–2

Recher, H., Gowing, G. and Armstrong, T. (1985) Causes and frequency of deaths among birds mist-netted for banding studies at 2 localities. *Australian Wildlife Research*, **12**, 321–326

Rivier, C. and Vale, W. (1984) Influence of CRF on reproductive functions in the rat. *Endocrinology*, **114**, 914–921

Rugh, D.J., Braham, H.W. and Miller, G.L. (1992) Method of photographic identification of bowhead whales, *Balaena mysticetus*. *Canadian Journal of Zoology*, **70**, 617–624

Sapolsky, R.M., Romero, L.M. and Munck, A.U. (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory and preparative actions. *Endocrine Reviews*, **21**, 55–89

Schaffner, C.M. and French, J.A. (2004) Behavioral and endocrine responses in male marmosets to the establishment of multi-male breeding groups: Evidence for non-monopolizing facultative polyandry. *International Journal of Primatology*, **25**, 709–732

Schultz, T.W., Rogers, K. and Aptula, A.O. (2009) Read-across to rank skin sensitization potential: subcategories for the Michael acceptor domain. *Contact Dermatitis*, **60**, 21–31

Teixeira, C.P., De Azevedo, C.S. and Mendl, M. (2007) Revisiting translocation and reintroduction programmes: the importance of considering stress. *Animal Behaviour*, **73**, 1–13

Wilkinson, D., Smith, G.C. and Delahay, R.J. (2004) A model of bovine tuberculosis in the badger *Meles meles*: an evaluation of different vaccination strategies. *Journal of Applied Ecology*, **41**, 492–501

Yokoyama, A.K. (1965) The effect of anaesthesia on milk yield and maintenance of lactation in the goat and rat. *Journal of Endocrinology*, **33**, 341–351