

Bullfrog *Lithobates catesbeianus* (Amphibia: Ranidae) tadpole diet: description and analysis for three invasive populations in Uruguay

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Abstract. The North American bullfrog, *Lithobates catesbeianus* is a dangerous invasive species that has been introduced worldwide for commercial and ornamental purposes. Although studies of the bullfrog's effects on invaded communities have been carried out, they have focused mostly on post-metamorphic stages. However, considering the experimental evidence and its ecological attributes, the *L. catesbeianus* tadpole could also have significant impact on invaded communities. The aim of this study was to perform a detailed qualitative and quantitative exploration of the *L. catesbeianus* larval diet. Gut content was studied and compared for three invaded locations in Uruguay (Departments of Canelones, Soriano and Cerro Largo). Although microscopic algae (*Spirogyra* spp., *Euglena* spp., *Closterium* spp., *Volvox* spp. and *Scenedesmus* spp.) were the most frequent prey, the diet was also made up of small invertebrates (especially rotifers) and eggs. Statistically significant differences in diet richness and prey identity were found among the three study sites, probably due to local conditions. One common feature among the three locations was the ingestion of prey with high protein levels, filamentous algae and animal prey. The high frequency of high-protein prey could explain the substantial growth rate of these larvae. Native tadpoles appear to have a much poorer diet than bullfrogs. We can thus assume that the *L. catesbeianus* larvae will be a major competitor and a potential predator for native communities. We propose that *L. catesbeianus* tadpoles may have significant effects on invaded communities, and should be considered in future research and managements plans.

Keywords: Aceguá, Bizcocho, pond, *Rana catesbeiana*, Rincon de Pando.

Introduction

The introduction and spread of exotic species is currently one of the greatest threats to global biodiversity. Biological invasions are not only responsible for different processes of diversity erosion, but often end up causing negative economic effects on ecosystem functions and human health. For these reasons, understanding the complex effects that exotic species generate on novel ecosystems is a conservation priority (Lockwood, Hoopes and Marchetti, 2007).

The North American bullfrog, *Lithobates catesbeianus* (Shaw, 1802) has been introduced around the world for commercial farming, biological control and ornamental purposes. As a consequence, this anuran has successfully invaded several sites in America, Asia and

Europe, where feral populations at establishment and expansion stages are found (Ficetola, Thuiller and Miaud, 2007). Because of its ecological attributes – phenotypic plasticity, high fecundity, large body size, broad diet and resistance to contaminants – *L. catesbeianus* potentially affects many native taxa through different mechanisms such as competition, predation, habitat modification and also indirectly, through the introduction of hitchhiker pathogens (Kupferberg, 1997; Pearl et al., 2004; Garner et al., 2006; Monello et al., 2006). Due to the negative effects on biodiversity and ecosystem functions, *L. catesbeianus* is considered by the IUCN as one of the most threatening invasive species worldwide (Lowe et al., 2000), and is related to the global phenomenon of amphibian decline (Blaustein and Kiesecker, 2002; Kats and Ferrer, 2003; Pearl et al., 2004).

Several researchers have issued a warning emphasizing the need to control or eradicate bullfrogs in invaded sites, as a way of preserving local biodiversity (Hulme, 2006; Adams and Pearl, 2007; Laufer et al., 2008; Kraus, 2009;

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Santos-Barrera et al., 2009). Because of the economic costs involved, as well as the complex ecological characteristics of bullfrogs (i.e. evasiveness, high fecundity rate and strong density-dependence response to harvest), this goal is difficult to achieve (Adams and Pearl, 2007). A first and essential step for the proper management of alien species is understanding their natural history (Lockwood, Hoopes and Marchetti, 2007). Although *L. catesbeianus* has been known to be a dangerous invasive species since the early 1900's and has been extensively studied, there are still uncertainties about its ecology in many of the recently invaded regions.

The study of its trophic relationships provides important information about the ecology of a species. Many studies have focused on the *L. catesbeianus* diet to understand its natural history and, like many other complex cycle species, the adult phase has been the most intensively studied (Boelter, 2004; Hiari, 2004; Pascual and Guerrero, 2008; Camargo Filho, 2009; Leivas et al., 2009; Teixeira da Silva et al., 2009). Research tends to focus on adults because the post-metamorphic stage is the most influential in amphibian population dynamics (Vonesh and De la Cruz, 2002). Even though the adult stage has the greatest effects on invaded communities, the *L. catesbeianus* larval stage shows some interesting features which suggest that it also plays an important role in the invasion process (Kupferberg, 1997; Boone, Little and Semlitsch, 2004). Its extremely large size (length from snout to tip of tail ranges from 76 to 170 mm) implies that the larval ecological attributes of this species are one of the most extreme for anurans (McNab, 2002). The long larval period is also noteworthy; the tadpole may remain at this stage through the winter without metamorphosing, for a period of more than a year (Govindarajulu, Price and Anholt, 2006). In addition, bullfrog tadpoles can represent an important fraction of the entire community biomass in invaded permanent lentic ponds (Bury and Whelan, 1984, e.g. Laufer et al., 2008).

Its oral structure – keratinized jaw sheaths and labial teeth, efficient filter systems – enables the bullfrog's exotrophic larva to graze efficiently on periphyton as well as on a wide variety of suspended algae (Seale and Beckvar, 1980; Pryor, 2003); its gut morphology and physiology indicate a typical herbivore with fermentative digestion (Pryor and Bjorndal, 2005). Most of the data about the feeding ecology of larval *L. catesbeianus* comes from experimental studies of interactions with other species (e.g. Seale and Beckvar, 1980; Kupferberg, 1997; Pryor, 2003; Govindarajulu, Price and Anholt, 2006), and aquaculture requirements tests (e.g. Carmona-Osalde et al., 1996). Although initially *L. catesbeianus* tadpoles had been considered an unselective basal resources consumer, the evidence reported by two recent isotopes and fatty acid field studies indicate that it may occupy a higher position in the trophic web (Schiesari, Werner and Kling, 2009; Whiles et al., 2010). Coprophagy seems to play an important role for these tadpoles, possibly assisting in digestion and increasing the availability of microorganisms (Steinwascher, 1978). The *L. catesbeianus* tadpole's high consumption rate (Pryor, 2003) of aquatic ecosystem resources can have significant effects on the performance of other larval frogs, such as reduced activity or survival, or smaller size at metamorphosis (Kupferberg, 1997). It is possible that bullfrog tadpoles are also affecting the invaded community structure through predation, although little work has been done to investigate this idea (Schiesari, Werner and Kling, 2009).

Despite the potential importance of *L. catesbeianus* larvae in aquatic communities' invasion processes, the studies of their diet in natural systems are scarce and poorly detailed. Knowing what the larvae ingest would provide an insight into their functional role in invaded communities through transformation of basal resources, i.e. changes in particle size of organic materials (Altig and McDiarmid, 1999) and removal of nutrients from primary production. Understanding more about the ecology of this in-

vasive species' tadpole, will enable us to improve activities for its control. The present study describes in detail the *L. catesbeianus* larval diet in three recently reported, invaded sites in Uruguay. In addition, we compared diet composition and richness among sites.

Materials and methods

We analyzed 77 individual tadpoles from three invaded sites in Uruguay; Aceguá (Cerro Largo Department; 31°53'49"S, 54°09'07"W, 218 m a.s.l., collected in August 2007, $n = 25$), Rincon de Pando (Canelones Department; 34°44'23"S, 55°55'30"W, 19 m a.s.l., collected in May 2005, $n = 30$) and Paraje Bizcocho (Soriano Department; 33°27'55"S, 58°10'08"W, 22 m a.s.l., collected in September 2007, $n = 22$). Bullfrog invasion focuses were reported for these three sites in Uruguay, originating from propagules released from commercial breeding farms. Feral populations of *L. catesbeianus* at the establishment stage were found in permanent lentic water bodies – relatively altered systems have depths of greater than 1 m – near the old farm facilities. In the invaded water bodies a strong community structure was observed, and an absence of native amphibians was noted (Laufer et al., 2008).

Tadpoles were collected with a hand trawl and then euthanized and fixed in 95% alcohol. In the laboratory, each tadpole was measured, staged and its gut contents was analyzed in detail. The total length (TL: from snout to tail tip) was measured with a digital caliper to the nearest of 0.1 cm. Analyzed larvae were between 25 to 39 according to the Gosner table of normal developmental stages (Gosner, 1960), which implies a wide developmental range.

Due to the length of the digestive tube of *L. catesbeianus* tadpoles, we only analyzed the content of the proximal four centimeters. Is in this segment, prey items can be found at an early stage of digestion and are therefore easily detected and identified (Rossa-Feres, Jim and Goncalves Fonseca, 2004; Pryor and Bjorndal, 2005). Gut content was suspended in water, on a glass slide and five subsamples of 0.5 ml were taken for identification. The number of subsamples was determined with the aim of species accumulation curves (Gotelli and Colwell, 2001). The entire sample was analyzed under a stereomicroscope (40×), paying special attention to classifying prey with the greatest possible taxonomic precision. Prey was determined according to Canter-Lund and Lund (1995) for algae, and Thorp and Covich (2001) for freshwater invertebrates. However, the information obtained here must be treated with caution as prey intake does not necessarily mean the capacity to digest it. This is common in tadpoles which eat a variety of material, but are not able to assimilate it all (Altig, Whiles and Taylor, 2007).

Data analysis

We explored differences between the sites in tadpole size, developmental stage, diet composition and richness using

the rarefaction procedure. Data analysis was performed using Statistica 6.0 (Stat Soft Inc.) and rarefaction procedure using EcoSim (Acquired Intelligence Inc.). Due to the non-normal distribution of the size and development data we used a non-parametric Kruskal-Wallis test to explore for statistically significant differences ($\alpha = 0.05$) (Sokal and Rohlf, 1994).

To explore the relationship between diet and collection site, we performed a Canonical Correspondence Analysis, (CCA; Gotelli and Ellison, 2004) this procedure detects consumption patterns, exploring which prey was related to each sampled site. For this analysis, we grouped species into taxonomic and functional groups. The group "Chlorophyta" was made up of *Oedogonium* spp., *Pediastrum* spp., *Scenedesmus* spp., *Euastrum* spp., *Staurastrum* spp., *Zygnema* spp., *Staurodesmus* spp., *Closterium* spp., *Cosmarium* spp. and *Volvox* spp.; "Cyanophyta" included *Anabaena* spp., *Oscillatoria* spp., and *Spirulina* spp.; "Euglenophyta" was made up of *Euglena* spp.; "Heterokontophyceae" by *Nitzschia* spp., *Dinobryon* spp., *Asterionella* spp., *Navicula* spp. and *Pinnularia* spp.; "Other animals" included Acari, Ancyliidae, Amphipoda, Calanoida, Nematoda, Otracoda, Hemiptera, Ephemeroptera, fish scales, Chironomid larvae and unidentified larvae. Further highly frequent groups were "Rotifers" and "Eggs". Differences among sites in tadpole diet richness were explored using the rarefaction procedure. This method estimates the expected richness of a series of subsamples from the prey abundance distribution data (Gotelli and Graves, 1996). The rarefaction curves allowed us to compare samples of different sizes taking into account rare species (Magurran, 1988) and obtain more reliable results than we could have obtained from other diversity indexes (Colwell, Mao and Chng, 2004). Statistical differences in site richness rarefaction curves were compared using 95% confidence intervals.

Results

Tadpoles collected at different sites showed significant differences in body size and developmental stage. The Kruskal-Wallis test showed statistically significant differences between samples in TL ($h = 33.0$, $d.f. = 2$, $P < 0.001$) and Gosner's stage ($h = 41.7$, $d.f. = 2$, $P < 0.001$). On average the individuals from Aceguá were larger and more highly developed. It should be noted that the sample from Rincon de Pando showed higher variability in size and development, when compared to the other sites.

With respect to the diet, we observed that microscopic algae were the numerically dominant and most frequent prey in larvae stomachs. The primary producers consumed were mainly

Spirogyra spp., *Euglena* spp., *Closterium* spp., *Volvox* spp. and *Scenedesmus* spp. at the three study sites (table 1). The diet also included small invertebrates, specillary rotifers and unidentified eggs. Other animal prey were inverte-

brate larvae and fish scales. Considering the absence of other resistant fish structures, we can assume that adult fish were not preyed upon by *L. catesbeianus* tadpoles. Scales are typical sediment components of fish habitat, as previously reported for the studied sites (Laufer et al., 2008).

Table 1. North American Bullfrog (*Lithobates catesbeianus*) larval diet, at three sites in Uruguay (Aceguá, Bizcocho and Rincon de Pando). For each site the number of specimens studied, their mean total length, their Gosner developmental stage range, the total number of items recorded in gut analysis and the identity and percentage of each prey item is shown.

	Aceguá	Bizcocho	R. Pando
Analyzed specimens	26	22	29
Mean total length (cm)	11.0	8.3	9.4
Gosner stage range	31-37	25-31	25-39
Total prey	7325	2461	8938
Prey identity and percentage			
<i>Anabaena</i>	0.83	0.04	0.01
<i>Oscillatoria</i>	0.08		0.45
<i>Oedogonium</i>	4.59	6.95	1.04
<i>Spirulina</i>	0.01		
<i>Chroococcus</i>	1.22	0.04	0.01
<i>Spirogyra</i>	17.98	0.65	0.02
<i>Cosmarium</i>	0.98	0.04	0.03
<i>Scenedesmus</i>	16.07	0.57	0.04
<i>Volvox</i>	1.09	0.12	13.09
<i>Closterium</i>	16.70	2.68	0.26
<i>Pediastrum</i>	2.59	0.98	1.45
<i>Euastrum</i>	3.63	0.08	
<i>Staurastrum</i>	3.89	0.04	
<i>Zygnema</i>	0.82		
<i>Asterionella</i>	0.74		
<i>Navicula</i>	3.14	0.81	3.37
<i>Euglena</i>	1.88	0.33	54.40
<i>Dinobryon</i>	1.09		
<i>Nitzschia</i>	0.49		5.91
<i>Pinnularia</i>	3.45	0.73	1.96
<i>Staurodesmus</i>	2.98		
Cladocera	0.14		
Acari	0.08		
Calanoida	0.56	0.28	0.03
Amphipoda			0.01
Ancylidae	0.05	0.04	
Rotifera	1.54	34.17	0.28
Nematoda	0.45	0.41	0.03
Ostracoda	0.38	0.04	0.20
Non-identified larvae	0.01	0.16	0.04
Chironomidae larvae		0.20	
Fish scales	0.05	0.04	
Eggs	12.46	50.55	17.33
Ephemeroptera		0.04	
Hemiptera juvenile			0.01

Larvae diet varied in number and composition with collection site (table 1). In the Bizcocho specimens, the most frequent prey items were eggs and rotifers, and secondly *Oedogonium* spp. (Chlorophyta). The diet of tadpoles from Bizcocho was less abundant and had an intermediate level of richness when compared with the other two sites. The most representative prey for samples from Aceguá were *Scenedesmus* spp., *Spirogyra* spp. and *Closterium* spp. (Chlorophyta). The intestines of specimens from Aceguá showed a greater abundance of prey and higher richness than those from Bizcocho. Finally, the Rincon de Pando tadpoles' diet has the lowest values for diet richness, but had the greatest abundance. These specimens mostly ingested *Euglena* spp. (Euglenophyceae), *Volvox* spp. (Chlorophyta) and eggs (fig. 1).

The correspondence analysis supported these results for all sites indicating an important association between sites and prey composition. The first factor of this analysis (representing 52.2% of total system inertia) separates the Aceguá and Bizcocho samples from Rincon de Pando, and shows association of Aceguá with the consumption of Chlorophyta, Cyanophyta and other animals. Rincon de Pando specimens were mainly associated with Heterokontophyceae and Euglenophyceae (fig. 1). Finally, Bizcocho was mainly associated with the consumption of animal prey (mostly rotifers), while the other sites were associated with algal diet. For analysis, the study sites were spread out along the axis according to the frequency of prey items encountered. The second factor (47.8% of total system inertia) separates Aceguá sample from the other two sites.

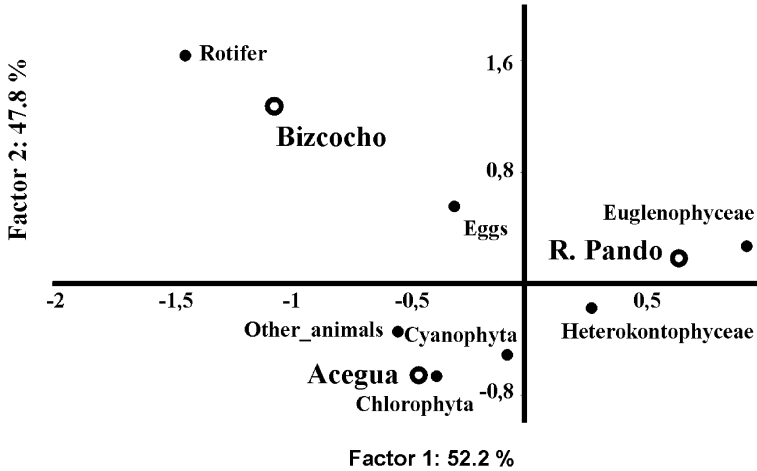


Figure 1. Scatterplot of factor scores from first two canonical correspondence analysis axes for the *L. catesbeianus* tadpoles diet. The three collection sites (○) are identified by the composition of items in diet (●). The first factor separates Rincon de Pando (right), from the other sites. The second factor, which also includes a significant percentage of inertia, separates Aceguá (below) from the other two sites (above).

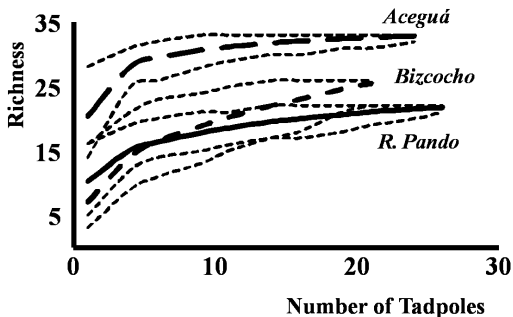


Figure 2. Comparison of diet richness for bullfrog (*Lithobates catesbeianus*) tadpoles collected at three sites in Uruguay (Aceguá, Bizcocho and Rincon de Pando), using rarefaction curves. Dashed fine lines indicate a 95% confidence interval for each site rarefaction curve.

Differences between sites were also supported by the rarefaction analysis which indicated that prey richness for the bullfrog larva was not constant (fig. 2). The confidence intervals show that the diet of the Aceguá tadpoles was much wider than at the other two sites (Bizcocho and Rincon de Pando). The richness curves also indicate that our sample size of 25 (Aceguá), 30 (Rincon de Pando) and 22 (Bizcocho) individuals was sufficient for obtaining estimates of diet composition.

Discussion

The success of an exotic species' establishment depends on the occurrence of novel interactions in the native community. The analysis of these trophic interactions during the establishment stage is crucial for understanding the mechanisms involved in the invasive process and its effects on community structure (Blumenthal, 2006). In this sense, our results are the first report of *L. catesbeianus* larvae diet for Uruguay and the region, with high taxonomic resolution. Even though there are other *L. catesbeianus* invasion records in Brazil and Argentina (Pereyra, Baldo and Krauczuk, 2006; Giovanelli, Haddad and Alexandrino, 2008; Akmentis and Cardozo, 2009; Barraso et al., 2009) dietary analysis has only been performed for Brazilian, and few Argentinian, adult specimens (Akmentis, Pereyra and Lescano, 2009; Da Silva et al., 2009). When a species with a complex life cycle is introduced, certain developmental stages (often poorly studied) become important because of their increasing local densities. This situation should be taken into consideration both when assessing their impact, and when proposing control measures (Kupferberg, 1997). Inter-

estingly, ecologists have not yet studied in detail the effects of the larval stage of the invasive *L. catesbeianus*. Considering its large size, biomass and permanence in aquatic systems, bullfrog tadpoles could cause a significant disturbance in native ecosystems. A complete understanding of the trophic ecology of invasive species is the baseline for further research, as well as a useful tool for developing proper management plans (Doubledee, Mueller and Nisbet, 2003).

Experimental evidence suggests that *L. catesbeianus* tadpoles are not strongly selective; they consume a wide variety of prey items, including different species and sizes of algae (Seale and Beckvar, 1980). The trophic profile presented in this study identifies the *L. catesbeianus* larvae as omnivorous and suggests that there is a preference for consuming prey from lower trophic levels (table 1; fig. 1). Nonetheless, the behavior could be considered opportunistic with great trophic plasticity, when considering the variations found, e.g. in prey availability (Whiles et al., 2010). Our observations revealed statistically significant differences in diet richness between collection sites, in the rarefaction. This could be due to ephemeral conditions of the different sites, or temporal variations of sampling, and to the plastic response of *L. catesbeianus* larvae to these variations. There were differences in diet richness associated with body size and developmental stage at individual level. Aceguá specimens were the largest and had the richest diet (fig. 2). The other two samples were similar in median body size and in diet richness. Having only three sample sites with few communities, and a relatively small sample size with limited ranges of body size, makes it difficult to draw conclusions.

Common dietary patterns detected at the three sites were the high consumption rate of eggs and Chlorophyta. This microalga can be an important nutrient source, as it is able to synthesize chlorophyll a and b pigments which are found in all photosynthetic plants (Lembi and Waaland, 1988). In addition, the pres-

ence of pirenoids (packed proteinaceous fibrils that store starch) and leucoplasts (specialized organelles that store starch) represent a great energy and protein source which favors tadpole development rates (Kupferberg, Marks and Power, 1994). Specifically, experimental studies have shown that increased protein intake results in higher larval growth (Carmona-Osalde, 1996). Kupferberg (1997) reported that this kind of algae favours timing and size at metamorphosis when consumed with epiphytic diatoms – which were also founded in the analyzed stomachs (table 1). Knowing the requirements of the early developmental stages of an invasive species can be an interesting instrument against its proliferation.

At the community scale, the introduction of an exotic generalist, which strongly reduces the available primary production, can have considerable effects. For example, an effect widely studied in the theoretical framework of biological invasions is the meltdown scenario, whereby one such organism facilitates further invasions through the removal of dominant native species (Adams, Pearl and Bury, 2003). Considering *L. catesbeianus* tadpole densities and their large digestive systems, its presence most likely affects the primary producers' assemblage.

Although the *L. catesbeianus* tadpole's diet was mainly dominated by microscopic algae (*Spirogyra* spp., *Closterium* spp., *Scenedesmus* spp., *Euglena* spp. and *Volvox* spp.), it also included small invertebrates (rotifers, calanoida, nematoda, ostracoda) and a large amount of eggs (table 1). Some of these prey items were encountered with high frequency, showing that *L. catesbeianus* tadpoles can act as predators (Alford, 1999; Mueller, Carpenter and Thornbrugh, 2006; Schiesari, Werner and Kling, 2009). Predation behavior can be context dependent, and may be beneficial for the bullfrog as it promotes faster larval growth (Whiles et al., 2010). Moreover, including animal prey in the tadpole diet considerably increases growth and development rates when compared with other conspecifics that do not consume ani-

mal material (Heinen and Abdella, 2005). Preying on other species could have a significant effect on the exclusion of these species from the invaded sites. Amphibian larvae can exclude other species from breeding in a body of water by preying on their eggs (Kiesecker and Blaustein, 1997, 1998; Kupferberg, 1997; Kiesecker, Blaustein and Miller, 2001). This reinforces the importance of including the different phases of an invasive species' life cycle when studying community structuring mechanisms. Future research should explore the idea that *L. catesbeianus* larvae, just like the adult bullfrog, could act as a strong regulator of community structure through the removal of primary producers or consumption of the eggs of other amphibian species.

There is a need to understand the role of exotrophic anuran larvae in water communities by detailed qualitative studies (Alford, 1999; Petranka and Kennedy, 1999; Boone, Little and Semlitsch, 2004; Smith, 2005; Kupferberg, 2006; Monello et al., 2006; Mueller, 2006; Altig, Whiles and Taylor, 2007). The evidence suggests that tadpoles can be an important factor in the trophic pathway of aquatic basal resources such as algae and detritus in food webs, and may also be an important predator in these ecosystems (Pryor, 2003; Schiesari, Werner and Kling, 2009). The importance of the frog's larvae should be considered in anuran invasions (Crossland, 2000). For example, available evidence shows that most native tadpoles in the region under study have a poorer diet than that observed for the exotic *L. catesbeianus* in the present study (Rossa-Feres, Jim and Goncalves Fonseca, 2004; Echeverría et al., 2007). We can thus assume that *L. catesbeianus* larvae will be a potential competitor and a predator on native communities. This should be analyzed in future studies considering not only the prey identity but also its quantity, combined with the significant biomass of *L. catesbeianus* larvae. Further research into the larval stages of exotic anurans could help to understand the strong observed effect at the invaded communities.

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