NORTH-WESTERN JOURNAL OF ZOOLOGY 17 (2): 196-203
Article No.: e211502

http://biozoojournals.ro/nwjz/index.html

American bullfrog (*Lithobates catesbeianus*) diet in Uruguay compared with other invasive populations in Southern South America

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Received: 22. May 2020 / Accepted: 25. February 2021 / Available online: 25. February 2021 / Printed: June 2021

Abstract. Between 2000–2020, more than ten new populations of the invasive American bullfrog (*Lithobates catesbeianus*) have been reported in the southern cone of South America. We studied the stomach contents of 126 bullfrogs from a population at an early invasion stage in Uruguay (Aceguá, Cerro Largo Department). We observed a rich diet, with extensive prey volume range (1 mm^3 to more than 7 000 mm^3); the most frequent items were Hymenoptera (19.6%), Coleoptera (16.4%), Amphipoda (13.3%), Anura (8.9%) and Heteroptera (8.7%). Despite some overlap, differences were observed in volume ($\chi 2 = 54.6$, $p < 0.001$, d.f. = 2) and prey quantity (F = 8.1, $p < 0.001$, d.f. = 79) between males, females, and juveniles. Juveniles showed significantly higher consumption of terrestrial prey by count (82% of their total ingestion) than adults (29% for males and 32% for females) ($\chi 2 = 28.5$, $p < 0.001$, d.f. = 2). Adults, especially females, showed a high frequency of cannibalism (33% of their total ingestion; $\chi 2 = 20.9$, $p < 0.001$, d.f. = 2). Comparing our data with other bullfrog regional studies, we found great plasticity in trophic habits and differences in the incidence of cannibalism (higher incidence in the populations of Aceguá, Uruguay, and Buenos Aires, Argentina). These differences could be related to local biodiversity, but also could be affected by the invasion phase. Cannibalism frequency was higher in small bullfrog populations, where it could be favoring the establishment success. This shift in foraging strategies during the invasion process had been insufficiently evaluated in amphibians. Knowing the ecological determinants for the invasion by bullfrogs can be useful to the development of management strategies.

Key words: cannibalism, feral population, gut contents, *Rana catesbeiana*, trophic ecology.

Introduction

Biological invasions are one of the main causes for global biodiversity loss, especially in aquatic systems (Sala et al. 2000, Bailey et al. 2020). Freshwater aquatic systems are disproportionately more prone to suffer negative impacts from invasive species than terrestrial systems (Ricciardi & MacIsaac 2011). The characteristics of freshwater ecosystems predispose them to severe, although not very visible, impacts of invasive alien species (Moorhouse & Macdonald 2015). Far from being controlled, this phenomenon is expected to increase in the coming decades because it has not been possible to reduce the introduction rate of exotic species globally (Seebens et al. 2017).

The American bullfrog *Lithobates catesbeianus* (Shaw 1802), one of the most widespread invasive vertebrates in the world (Kraus 2009), negatively affects native communities (especially amphibians), through predation, competition, habitat alteration and transmission of diseases (e.g. Kraus 2009, Both et al. 2011, Both & Grant 2012, Miaud et al. 2016). This voracious predator of large body size (maximum snout-vent length = 200 mm), is native to eastern North America and has been introduced since the early 1900s in different regions around the globe for aquaculture purposes (Collins & Crump 2009). This introduction path explains most of the bullfrog invasive populations on a global scale (Kraus 2009).

The introduction of the bullfrog to Southern South America (south of the 26th parallel) was led by farms producing frogs for human consumption in Brazil. The first introductions occurred in 1935 in the State of Rio de Janeiro, where bullfrog farms are still operating (Pahor-Filho et al. 2019). This industry growth generated a strong invasion of the bullfrog in natural ecosystems (Both et al. 2011). In Argentina and Uruguay, the frog farming industry was rapidly developed, but it failed economically shortly after its establishment. Multiple introductions in the wild since the 1970s and 1980s led to the establishment of several invasive populations that pose various environmental risks for local biodiversity (Laufer et al. 2008, 2018, Akmentins et al. 2009, Akmentins & Cardozo 2010, Nori et al. 2011, Sanabria et al. 2011a, b). According to a niche models evaluation (Barbosa et al. 2017), the region of Southern South America presents optimal climatic conditions for the bullfrog. In addition, the landscape modification due to agricultural activity generates a high density of permanent lentic environments, highly suitable for this amphibian (Minowa et al. 2008, Liu et al. 2016).

Existing evidence from Southern South America shows that the bullfrog invasion affects the structure of aquatic communities (Laufer et al. 2008, Batista et al. 2015, Laufer & Gobel 2017, Oda et al. 2019), produces acoustic niche interference (Both & Grant 2012) and spreads diseases (Schloegel et al. 2010). Bullfrog larvae are able to ingest a great diversity of algae primary producers, invertebrates and undetermined eggs (Ruibal & Laufer 2012), while adults have a more varied diet (studied in Argentina by Akmentins et al. 2009, Barrasso et al. 2009, Quiroga et al. 2015, and in Brazil by Boelter & Cechin 2007, Leivas et al. 2012a, Silva et al. 2009, 2010, 2011, 2016). Quiroga et al. (2015) demonstrated the ability of the bullfrog to establish in extreme environments (streams in the high altitude Andean desert), adjusting its diet to the low local species richness.

Knowledge about the trophic ecology of an exotic predator in a new environment is an essential information to understand its potential effects (Solé & Rödder 2010). Studying diet helps us to understand how an invader is performing in the acquisition of food resources, which sustain its populations and could affect native species (Kats & Ferrer 2003). In this context, stomach content analyses can provide essential data about the novel functional connections with different native taxa and energy paths, which cannot be directly inferred from observational studies or evaluations of community structures. Therefore, information on the diet of an invader is necessary for the understanding of its effects and for planning its management (Dick et al. 2013).

Empirical evidence showed a great dietary plasticity in post-metamorphic individuals of *L. catesbeianus*, consuming any live animal they are capable of ingesting, including conspecifics and other amphibians (Bury & Whelan 1984, Adams & Pearl 2007). There is no specific evaluation of the wild populations in Uruguay, although the bullfrog diet has been studied in neighboring countries (e.g. Silva et al. 2009, Quiroga et al. 2015). Thus, the objective of the present study was to evaluate the post-metamorphic bullfrog diet in an Uruguayan population. For this, the stomach content of bullfrogs from a population at the early stage of invasion in the locality of Aceguá (Cerro Largo Department) was analyzed. We compared our results with data from other regional studies. We hypothesized that there should be differences in the composition of the diet, associated with the local availability of prey and with the phase of invasion.

Material and Methods

Field sampling

We collected individuals in non-systematic surveys, in the locality of Aceguá (31°53′49″S, 54°09′07″W; datum WGS1984; Cerro Largo Department, Uruguay), from 2007 to 2013. In this locality, a wild population of bullfrog in the establishment phase was detected in 2007, restricted approximately 1 200 m around the site were an old bullfrog farm (closed in the 2000s) was located (Laufer & Gobel 2017, Laufer et al. 2018). Post-metamorphic bullfrogs were collected by hand at night (21:00 h to 00:00 h), during the breeding season from November to December, in a permanent lentic freshwater system consisting of nine ponds and artificial water reservoirs. These water bodies had an average surface of approximately 2 647 m^2, with an average distance between ponds of 1 690 m. They had pH = 7.4 (range: 6.3–8.5), conductivity = 95.9 µS/cm (62–151), dissolved oxygen = 7.1 mg/l (1.1–10.4), and 39% (5–100) of their surface was covered by floating or emergent macrophytes; their maximum depth was 2 m, and they were surrounded by natural grasslands in extensive cattle farms. Native amphibian species found at this site were *Leptodactylus luctator*, *L. latinasus*, *L. gracilis*, *L. mystacinus*, *Dendropsophus minutus*, *D. sanborni*, *Phyllomedusa iheringii*, *Boana pulchella*, *Scinax squalirostris*, *S. granulatus*, *Julianus uruguayus*, *Ololygon aromothyella*, *Pseudis minuta*, *Pseudopaludicola falcipes*, *Limnomedusa macroglossa*, *Odontophrynus americanus*, *Physalaemus biligonigerus*, *P. riograndensis* and *Elachistocleis bicolor*. Further information on the location and description of these sites is provided in Laufer et al. (2018) and Gobel et al. (2019).

Bullfrogs were located by staff trained to identify the species, along the shoreline or in water. Once captured by hand, frogs were immediately sacrificed with an overdose of Eugenol and fixed in 10% formaldehyde, following national and international animal welfare regulations (Leary et al. 2013). Then, at the laboratory of the Museo Nacional de Historia Natural, Montevideo (MNHN), individuals were measured with a digital caliper from the snout to the cloaca (SVL, snout-vent length, to the nearest 0.01 mm). Individuals were classified either as juveniles (J, without obvious sexually dimorphic characters, with SVL lower than the minimum size of male bullfrogs, 55 mm; following Wang et al. 2008), adult females (F, sexed based on secondary sexual traits, i.e. relative size of the external tympanum, coloration and presence of swollen thumbs; Howard 1981), or adult males (M). Although juveniles did not present external secondary sexual characters, it should be considered that they could include some sub-adults according to reports for Southern Brazil (Kaefer et al. 2007, Leivas et al. 2012b).

Stomach content analysis

The analysis of stomach contents was performed after dissection and extraction of the stomach from fixed specimens. The contents were analyzed in a Petri dish under a binocular magnifying glass (Nikon SMZ-445), and preserved in separate vials for each individual of 70% ethanol. Voucher bullfrog and stomach contents were housed at the herpetological collection of the MNHN (catalog numbers MNHN 4014–16, 4018, 4021–23, 4025, 4040, 4041–47, 4062, 4050, 4065–67, 4093, 4108, 4109).

Prey items were identified to the lowest possible taxonomic level, with the aid of regional identification keys and species lists (Mugnai et al. 2010, Teixeira de Mello et al. 2011, Gobel et al. 2013). The ingestion of plants and inorganic substances was considered accidental and not included in further analyses. Each prey was classified according to its natural history into "aquatic" and "non-aquatic" classes, based on our field observations and bibliography (e.g. Mugnai et al. 2010). For each prey, measurements of length and width were taken with a digital caliper and then its volume was obtained by the equation for an prolate ellipsoid: $\text{volume} = 4/3 \times \pi \times (\text{length}/2) \times (\text{width}/2)^2$ (Dunham 1983).

Statistical analyses

The index of relative importance (IRI) for each prey was calculated using the formula: $IRI = (N+V) \times F$, where N = numerical percentage, V = volumetric percentage, and F = frequency of occurrence in stomach. IRI values are suitable for ranking the relative importance of food items, considering that the higher the IRI, the higher the importance of a given prey category (Pinkas et al. 1971).

Differences in body size (SVL) between demographic groups were evaluated by a Kruskal-Wallis non-parametric test (due to the non-normality in the data). Statistical significance was tested by Chi-square, and then a post hoc between paired demographic groups. Differences in prey number (total quantity of individual items per stomach), between demographic groups were tested using a General Linear Model (GLM), F-statistic analysis (family Poisson, commonly used for count variables; Logan 2011). Total ingested prey volume per stomach was tested by a Kruskal-Wallis non-parametric test, by Chi-square, and then a post hoc between paired demographic groups. Then, the proportion of aquatic prey items (total number of aquatic items / total ingested items, per stomach) and aquatic prey volume (total volume of aquatic items / total ingested volume, per stomach) was compared between demographic groups by a Kruskal-Wallis non-parametric test, by Chi-square, and then a post hoc between pared demographic groups (Logan 2011).

Differences in prey richness (number of different prey per stomach), between demographic groups were tested using a GLM, F-statistic analysis (family Poisson). Prey richness was compared among demographic groups by the rarefaction procedure using the R package iNEXT (Hsieh et al. 2016). This analysis is a robust method to compare disparate samples, and allows evaluating the completeness of the data (Chao & Jost 2012).

Trophic diversity was calculated using Hurlbert's probability of interspecific encounter index (PIE) (Hurlbert 1971). This index ranges from zero to one, and refers to the probability that two randomly selected individual prey will be of different taxa. We calculated the PIE index for each demographic group, using the R package benthos (van Loon et al. 2015).

We calculated feeding overlaps between adults and juveniles, and between males and females, using Pianka's index (Pianka 1974). This index evaluates the overlap as the consumption of equally available common prey, between two groups (Krebs 1999). To verify the presence of non-random patterns, we calculated and tested our results against null models. We used 5000 randomizations to create pseudo communities, and then statistically compared the mean niche overlap values for A-J and M-F, in these randomized communities with the observed data matrix (algorithm ra3). Interspecific food partitioning might be occurring if the observed mean overlap values are significantly lower than those expected by the null model, whereas similar foraging patterns (corresponding to niche overlap) have higher values than those expected by chance (Winemiller & Pianka 1990). We calculated this Pianka's index using the R package spaa (Zhang 2016), and we tested the null models using the R package EcoSimR (Gotelli & McGill 2006, Gotelli et al. 2015). Regarding statistical significance, the p value obtained in the simulations reflects the probability that the observed value is greater than, or equal to the mean of the simulations performed with the ra3 algorithm.

The incidence of cannibalism, defined as the number of conspecific prey items (tadpole, juvenile or adult) per stomach, was evaluated for demographic groups (J, F and M) and for body sizes (SVL). Differences in the incidence of cannibalism (number of conspecifics per stomach) between demographic groups (J, F, M) were analyzed by a Kruskal-Wallis non-parametric test, by Chi-square, and then a post hoc between paired demographic groups. Finally, to evaluate the relationship of the incidence of cannibalism with the individual body size (SVL), we used a GLM (family binomial). The response variable was presence-absence of cannibalism and SVL was the explanatory variable. All analyses were performed excluding individuals with empty stomachs. All analyses were performed with the software R, considering an $\alpha = 0.05$ (Logan 2011, R Core Team 2019).

We compared our data with other regional diet studies: two from Argentina (9 de Julio, Buenos Aires Province, Barrasso et al. 2009; Calingasta, San Juan Province, Quiroga et al. 2015), and two studies from Brazil's state of Minas Gerais: campus of the Universidade Federal de Viçosa and Represa do Belvedere, in the municipality of Viçosa, and Santo Antônio do Glória, in the municipality of Vieiras (Silva et al. 2009, 2016). For this, each publication was reviewed and diet data were extracted, i.e. the numerical frequency of each food item for adults and juveniles. Adults were analyzed without discriminating between sexes because some studies did not separate males and females. We did not include other diet reports (e.g. Boelter & Cechin 2007, Akmentins et al. 2009), because they have anecdotal or non-comparable prey taxonomic data. The comparisons were made through a Correspondence Analysis and their statistical significance was analyzed through a Chi-square test (Legendre & Legendre 2012).

Results

The analyzed sample was composed of 51 juveniles (10 with empty stomachs), 23 adult females, and 29 adult males (2 with empty stomachs; Table 1). Mean adult SVL did not differ between sexes (males mean SVL = 119.2 ± 35.3 mm Standard Deviation; females mean SVL = 128.6 ± 32.3 mm), but were significantly greater than those of juveniles (SVL = 40.0 ± 8.7 mm; $\chi 2 = 57.2$, $p < 0.001$, d.f.= 2; Fig. 1A). Stomach contents showed a varied diet, including decapods, gastropods, arachnids, insects, and vertebrates (Table 1). Among the main items in numerical proportion, we observed Hymenoptera (19.6%), Coleoptera (16.4%), Amphipoda (13.3%), Anura (8.9%), Heteroptera (8.7%), Odonata (4.6%), Ephemeroptera (4.6%), Diptera (4.3%), Araneae (3.9%) and Orthoptera (3.4%). In addition, we observed a great variation in the magnitude of total volume of prey, with a range from 1 mm^3 to more than 7 000 mm^3 (Table 1).

On average, males consumed twice as many prey items than females and juveniles (F = 8.1, $p < 0.001$, d.f. = 79; Fig. 1B). Females consumed a mean volume estimated at 4 986 ± 7 081 mm^3 that did not differ statistically from the volume ingested by males (7 829 ± 14 843 mm^3). A significant difference between juveniles and adults (F and M) was found ($\chi 2 = 54.6$, $p < 0.001$, d.f. = 2, Fig. 1C). Juveniles ingested a mean volume prey of 32 ± 77 mm^3.

Both terrestrial and aquatic prey items were observed in the diet of *L. catesbeianus*. More than half of the prey recorded in adult stomachs (both by number of items and volume), came from the aquatic ecosystem. In contrast, juveniles showed significantly higher consumption of terrestrial prey, reaching three-quarters of their total ingestion (by number, $\chi 2 = 28.5$, $p < 0.001$, d.f.= 2, and by volume, $\chi 2 = 24.9$, $p < 0.001$, d.f.= 2; Fig. 1D, E).

Prey richness was higher in adults than in juveniles (ANOVA, F = 16.3, p = 0.001, d.f. = 79; Fig. 1F), but this difference were not detected by the rarefaction analysis, considering the overlap of the 95% confidence intervals. Rarefaction results indicate that the differences found were due to the sample size (Fig. 2). The diet of the three demographic groups presented comparably high trophic diversity (Hurlbert's index $PIE_M = 0.96$, $PIE_F = 0.95$, $PIE_J = 0.94$). The highest niche overlap was observed between adults and juveniles (Pianka's index observed = 0.77, estimated = 0.28, $p_{obs>est} = 1.00$), whereas the lowest value was observed between males and females (Pianka's index = 0.59, estimated = 0.32, $p_{obs>est} = 0.977$).

Finally, we observed cannibalism only in adults. The proportion of conspecific prey, both tadpoles and juveniles, was 0.33 ± 0.43 for females and 0.18 ± 0.31 for males. The Kruskal-Wallis non-parametric test, showed statistically significant differences between females and juveniles ($\chi 2 = 20.9$, $p < 0.001$, d.f.= 2, Fig. 3A). Using a binomial GLM model, we were able to find the relationship between cannibalism ratio and SVL (explained deviance = 0.6%, adjusted quality = 0.76). This model predicted that cannibalism occurs in individuals over 130 mm in SVL and increases strongly with size (Fig. 3B).

For the comparison of regional data, each reported item was assigned to the following major groups: Ephemeroptera, Diplopoda, Orthoptera, Mollusca, Other Crustaceans, Decapoda, Lepidoptera, Diptera, Hemiptera, Odonata, Arachnida, Coleoptera, Hymenoptera, native vertebrates and *L. catesbeianus* (tadpoles or post-metamorphs). The Correspondence Analysis significantly discriminated the diets from the different bullfrog populations, in relation to their prey frequencies (correlation coefficient = 1.107, $\chi 2 = 3071.13$, d.f. = 140, $p < 0.0001$, Fig. 4). Meanwhile, we observed that the first axis (with 38.7% of the variance in the data) separated the San Juan Province population (mainly associated with consumption of Hymenoptera, Mollusca, and Decapoda) from the rest of the populations (associated with a greater prey richness). The second axis (with 23.1% of the variance in the data) separated the populations from Buenos Aires

Table 1. Bullfrog *L. catesbeianus* diet in Aceguá, Cerro Largo Department, Uruguay. Gut content is presented for adults and juveniles. Each prey is presented with its taxonomy and its habits: aquatic (A) or terrestrial (T). n = number of individuals, Vol = estimated volume, IRI = index of relative importance, L = larva, A = adult. The volume data that could not be obtained appear as na (not available).

Prey					Juveniles			Adults		
Class	Order	Family	Genus / Species	Terrestrial/ Aquatic	n	Vol (mm^3)	IRI	n	Vol (mm^3)	IRI
Malacostraca	Decapoda	Trychodactylidae		A				3	6 854.58	3.33
Malacostraca	Amphipoda			A	32	188.73	77.44	23	102.29	10.11
Malacostraca	Isopoda			T	2	128.95	3.63	1	37.75	0.15
Branchiopoda	Cladocera			A				8	na	0.58
Ostracoda				A				2	na	0.15
Diplopoda				T				1	123.37	0.17
Gastropoda	Pulmonata	Ancylidae		A				1	0.18	0.07
Arachnida	Araneae			T	8	25.75	18.98	8	140.73	8.93
Arachnida	Acari			A	1	0.15	0.13	2	0.033	0.29
Arachnida	Opiliones			T				1	247.49	0.19
Insecta	Blattaria	Blattellidae		T	1	106.21	2.82			
Insecta	Coleoptera	Curculionidae		A	2	14.86	1.76	16	187.69	23.65
Insecta	Coleoptera	Dytiscidae (A)		A				8	4 613.52	9.96
Insecta	Coleoptera	Dytiscidae (L)		A	1	0.48	0.14	1	0.032	0.07
Insecta	Coleoptera	Hydrophilidae (A)		A				14	4 787.85	31.87
Insecta	Coleoptera	Staphylinidae		T	1	0.18	0.13			
Insecta	Coleoptera	Scarabaeidae		T	1	122.12	3.20	10	4 222.9	11.07
Insecta	Coleoptera	Grynidae		A				1	468.94	0.23
Insecta	Coleoptera (L)			A	1	48.35	1.42			
Insecta	Coleoptera			na	6	52.15	14.12	6	1 644.28	5.84
Insecta	Collembola			na				1	na	0.07
Insecta	Diptera	Cyclorrapha		T				1	5.86	0.15
Insecta	Diptera	Nematocera		T	5	21.68	3.66			
Insecta	Diptera	Tabanidae		T				1	na	0.07
Insecta	Diptera (L)			A				1	5.86	0.15
Insecta	Diptera (pupa)			A				2	0.18	0.15
Insecta	Diptera			T	7	25.97	13.50	1	3.96	0.15
Insecta	Ephemeroptera (A)			T	18	60.21	24.62	1	105.84	0.16
Insecta	Hemiptera	Cercopidae		T	3	5.09	0.91			
Insecta	Heteroptera	Belostomatidae		A	2	21.23	1.55	25	12 378.95	87.88
Insecta	Heteroptera	Corixidae		A	1	25.99	0.89			
Insecta	Heteroptera	Notonectidae		A	1	2.43	0.32	5	122.39	1.88
Insecta	Heteroptera			T	2	2.56	0.88			
Insecta	Homoptera			T	1	27.00	0.91			
Insecta	Hymenoptera	Apidae		T	1	5.92	0.40	12	842.98	13.29
Insecta	Hymenoptera	Formicidae	*Acromyrmex*	T	11	46.38	11.98	25	105.34	21.97
Insecta	Hymenoptera	Formicidae	*Brachymyrmex*	T				1	0.73	0.07
Insecta	Hymenoptera	Formicidae	*Pheidole*	T				2	2.27	0.44
Insecta	Hymenoptera	Formicidae	*Solenopsis*	T				2	4.85	0.58
Insecta	Hymenoptera	Formicidae		T	21	89.23	49.64	3	3.75	1.09
Insecta	Hymenoptera	Vespidae		T				2	333.64	0.70
Insecta	Hymenoptera			T				1	1.20	0.15
Insecta	Isoptera	Termitidae		T	1	6.09	0.41			
Insecta	Lepidoptera (L)			T				2	483.98	0.38
Insecta	Lepidoptera (A)			T	1	4.74	0.38			
Insecta	Odonata	Anisoptera (A)		T	1	5.13	0.38			
Insecta	Odonata	Anisoptera (L)		A	1	477.53	11.75	9	4031.78	12.20
Insecta	Odonata	Zigoptera (A)		T				4	71.80	1.19
Insecta	Odonata (A)			T	2	6.26	0.67	2	na	0.29
Insecta	Orthoptera	Gryllidae		T	4	106.84	14.47	2	227.55	0.66
Insecta	Orthoptera	Gryllotalpidae		T				5	1 029.29	2.74
Insecta	Orthoptera			T	2	0.70	0.54	1	459.40	0.23
Insecta	Thysanura			T				1	170.09	0.18
Insecta				na	9	0.77	9.49	2	na	0.29
Actinopterygii	Characiformes	Characidae	*Astyanax* sp.	A				6	8 459.49	7.17
Actinopterygii	Characiformes			A				1	3 320.26	0.74
Amphibia	Anura	Hylidae	*Boana pulchella* (L)	A				2	4 337.06	1.07
Amphibia	Anura	Ranidae	*L. catesbeianus* (L)	A				23	74 906.63	167.64
Amphibia	Anura	Ranidae	*L. catesbeianus* (A)	A				11	7 7421.66	77.33
Amphibia	Anura			T				1	2 526.60	0.60

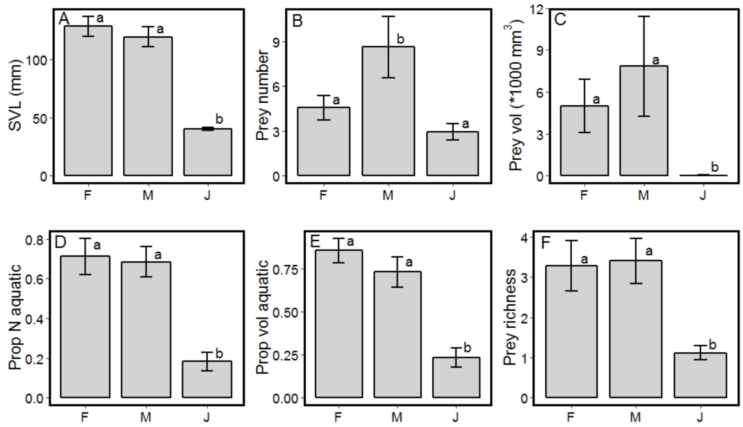

Figure 1. Bullfrog *L. catesbeianus* stomachs contents and differences between demographic groups (Females, Males and Juveniles) in mean body size, measured as snout vent length = SVL (A), mean prey number = Prey number (B), mean prey volume = Prey vol (C), mean proportion of number of aquatic prey = Prop N aquatic (D), mean proportion of volume of aquatic prey = Prop vol aquatic (E), and mean prey richness = Prey richness (F). The bars show the standard error. Statistically significant differences between demographic groups are indicated with different lowercase letters. The absence of statistical significant differences is indicated as n.s.

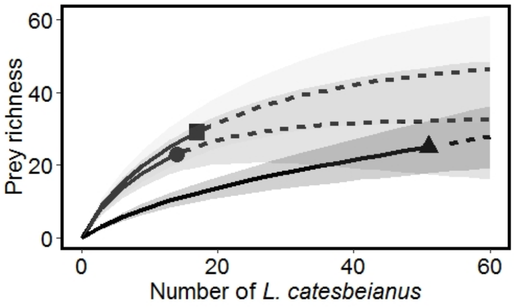

Figure 2. Prey richness analyzed by rarefaction procedure for Bullfrog *L. catesbeianus* stomachs from Aceguá, Uruguay. The result of rarefaction is shown for females (circle), males (square) and juveniles (triangle). The continuous lines are the result of the interpolation made by the rarefaction analysis, while the dotted lines are the extrapolation. The shaded area represents the 95% confidence intervals.

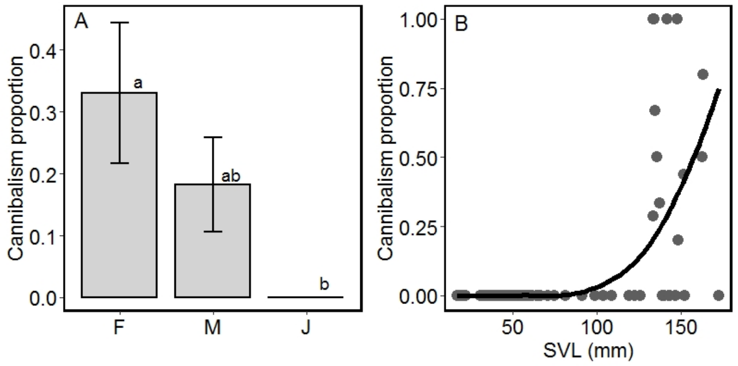

Figure 3. Cannibalism proportion in the Bullfrog from Aceguá, Uruguay (= Cannibalism prop). Data are provided for demographic groups (A) and for individual SVL (B). In (A), the bars show the standard error. Statistically significant differences between demographic groups are indicated with different lowercase letters. In (B), the prediction of the binomial model was included.

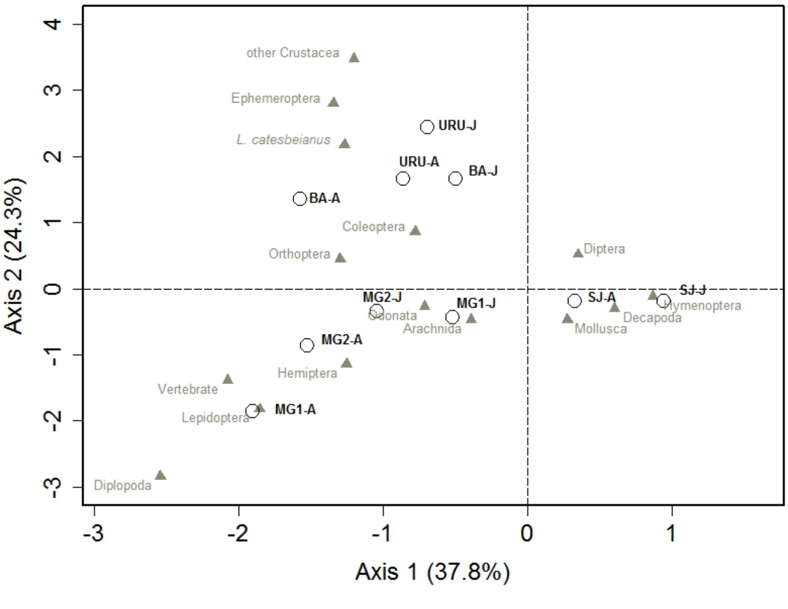

Figure 4. Correspondence analysis for the frequency data of diet of this study (URU) and those of San Juan (SJ, Quiroga el al. 2015), Province of Buenos Aires (BA, Barrasso et al. 2009), Argentina, and those of Minas Gerais, Brazil (MG1, MG2, MG3, Silva et al. 2009, 2010, 2016). For each data set, it is discriminated between adults (A) and juveniles (J). Study sites are represented by circles and prey by triangles.

Province and Uruguay (associated with consumption of *L. catesbeianus* and crustaceans) from the rest of the populations from south-eastern Brazil (associated with consumption of Diplopoda, Lepidoptera, and native vertebrates).

Discussion

Our findings, the first diet evidence for post-metamorphic *L. catesbeianus* in Uruguay, showed great plasticity, related to the exploitation of resources from both aquatic and terrestrial environments. Despite the species' aquatic habits, bullfrog populations seem to be partially supported by terrestrial prey. In fact, juvenile bullfrogs are known to exploit resources from shallow temporary water bodies and vegetated areas adjacent their aquatic habitats (Gahl et al. 2009). Prey diversity, belonging to different environments and trophic levels, shows that bullfrogs could be acting as a novel generalist top predator within local frog assemblages (Bury & Whelan 1984, Hirai 2004, Wang et al. 2008, Jancowski & Orchard 2013). This integration of different pathways and food webs could sustain the populations of this large-bodied anuran (Woodward & Hildrew 2001, Arim et al. 2010).

According to our observations, bullfrog feeding strategies change along its ontogeny. Despite some overlapping niches, we could identify strong differences between demographic groups, especially in diet composition and volume, between juveniles and adults. This shows the occurrence of different foraging strategies at each phase. Bissattini et al. (2019) showed that bullfrog juveniles overlap in diet composition with native anurans, suggesting a significant degree of competition for trophic resources.

Initially, bullfrog tadpoles feed on aquatic organisms (mostly primary producers) and detritus, incorporating small invertebrates and undetermined eggs (Schiesari et al. 2009, Ruibal & Laufer 2012). Then, after metamorphosis juveniles consume a great proportion of terrestrial prey, increasing aquatic prey intake in the adult phase (e.g. Hirai 2004, Bissattini et al. 2019). The observed variations show the capacity of this species to use different resources, but the intake of mostly terrestrial prey by juveniles may also have other explanations. Juveniles frequently appear in terrestrial areas, probably to disperse, escape predation or cannibalism and even to thermoregulate (Lillywhite 1970, DeAngelis et al. 1980). This diet shift in juveniles could be possibly explained by the predation risk associated with the presence of conspecific adults inside the water bodies. As we observed in Uruguay, cannibalism seems to be a frequent strategy in adult bullfrogs, which could affect juveniles’ habitat selection and diet (Foster et al. 1988).

Cannibalism is a phenomenon widely reported in amphibians, with individual benefits in survival or reproduction (Bury & Whelan 1984, Polis & Myers 1985, Stuart & Painter 1993). It can be a strategy for the integration of major energetic pathways (Mayntz & Toft 2006), generating an important regulation of population structure and dynamics (Fox 1975, Polis 1981, Ziemba & Collins 1999, Park et al. 2005, Measey et al. 2015). This regulation has such a significant impact on the invasive populations of bullfrogs that Govindarajulu et al. (2005) concluded that the elimination of adults could lead to greater survival of the early post-metamorphic stages, explained by a reduction in cannibalism rate. Managing this behavior can lead to future development of control strategies for amphibian invasions (Crossland et al. 2012).

The comparison of diet studies in Southern South America showed the bullfrog’s great plasticity (Kraus 2009). This species is notorious for invading very different communities. Unlike the rest of the studies, the desert high Andean environment of Argentina, sustains bullfrogs with restricted prey diversity (Quiroga et al. 2015). In the correspondence analysis that population differs from the others in its prey composition and richness. In any case, the second axis of the analysis separates the populations of Buenos Aires and Uruguay from those from south-eastern Brazil (Minas Gerais). This could be due to geographical differences that affect prey richness and abundances (e.g. Vinson & Hawkins 2003, Villalobos et al. 2013), but also could be affected by differences in bullfrog invasion stage. While the Brazilian populations were in an expansion phase (Both et al. 2011), those of Uruguay and Buenos Aires were probably at an early establishment stage (Barrasso et al. 2009, Laufer et al. 2018).

While Brazilian populations consumed a greater amount of native anurans (Boelter & Cechin 2007, Silva et al. 2009, 2010, 2016), populations from Buenos Aires and Uruguay were mostly cannibalistic. Jancowski & Orchard (2013) reviewed worldwide bullfrog diet studies finding that cannibalism was a minor component of the diet, increasing only in the absence of alternative prey. These authors suggest that cannibalism remains an option that would be of variable importance from site to site, season to season, and year to year. They also considered that cannibalism should be important when bullfrog diet would drive down native amphibian abundances. Our regional analysis is plausible evidence towards understanding the role of cannibalism during the different phases of bullfrog invasion. While at early stages conspecific consumption would be a strategy to access more resource paths, cannibalism would decrease during the expansion phase. In this phase, bullfrog densities would be lower (at the invasion front) and the encounter rate with native amphibians would be higher (Pizzatto & Shine 2008, Measey et al. 2015). Changes in foraging strategies and cannibalistic behavior have been identified in other invasive species and have been associated with the availability of prey and the invasion dynamics (e.g. Cottrell 2005, Carol et al. 2009, Brown et al. 2013). Understanding the differences in dietary strategies between ontogenic stages and population phases of an invader is key information for the development of successful control strategies.

Our study contributes with relevant data for management of bullfrog invasion in Southern South America and the related environmental risks. In addition, the Uruguayan environmental authorities should consider our results as a call for the need of an urgent effective control of the invasion of the bullfrog. In this sense, the recent 283/2020 Resolution of the Environment and Sustainable Development Ministry of Argentina, declaring to American bullfrogs as a harmful invasive species for the biodiversity is a promising example in the region.

Acknowledgement. GL and NG thank the Agencia Nacional de Investigación e Innovación (ANII) and the Programa de Desarrollo de las Ciencias Básicas (PEDECIBA), Uruguay, for their postgraduate grants. GL is a member of the Sistema Nacional de Investigadores (SNI), Uruguay. We all thank the local residents of the studied sites for their contributions during fieldwork. We are indebted to the Rufford Foundation for providing financial support for our research. GL and NG thank ANII for their scholarships (POS_NAC_GL_doctorado and POS_NAC_2015_1_109517). We thank the editor Yurii Kornilev, one anonymous reviewer, and Emanuel Teixeira da Silva for their valuable comments, which helped us to improve the manuscript.

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