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Invariant and vulnerable food web components after bullfrog invasion

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Abstract

Alien species introductions produce strong impacts on invaded communities, altering their structure, diversity and functioning. These impacts are interrelated with changes in food web architecture. However, the reorganization or robustness of food webs in the face of invasions is a phenomenon poorly considered in ecology. In this article, we analyze the effects of American bullfrog, *Lithobates catesbeianus*, invasion on the structure and function of invaded food webs. Specifically, we analyzed the integration of energetic channels by top predators, the relative use of alternative energetic paths by different functional groups and its dependence on body size among invaded and uninvaded communities, in Uruguay, Southamerica. The integration of energetic paths at high trophic positions by large body sized consumers was a pervasive feature of food webs among all studied ponds, in spite of turnover in top predators identity and large changes in communities composition. Bullfrog post-metamorphs presented high trophic positions, integrating the primary producers and detritus paths, acting as apex predators in invaded food webs. The bullfrog tadpoles presented intermediate positions and were associated with the detritivorous pathway. On the other hand, the relative importance of the primary producers and detritus as carbon sources assimilated into the biomass of fish and invertebrates was altered in invaded systems. The robustness in the integration pattern of energy channels is congruent with its proposed central role in the stability of food webs. These results advance the understanding of the effect of invasions on key structural features of food webs, notably underrepresented in the invasion literature.

Keywords: Pond, Aquatic community, Trophic path, Stable isotope analysis, *Lithobates catesbeianus*, *Rana catesbeiana*

Introduction

Identifying the structures and processes that confer stability to communities is a historical and central aim of ecology (Kondoh 2003; May 2006; Garay-Narváez et al. 2013). The central role that the structure of food webs plays in community stability was identified early in ecological studies (Pimm 1984). An important structural component in food webs is the dynamics and coupling of energy channels (Rooney et al. 2006, 2008; Arim et al. 2007, 2010; Rooney and McCann 2012; Potapov et al. 2019; Keppeler et al. 2021). Food webs present two energy flow pathways with contrasting properties, the primary producer (also called "green channel") and the detrital energy channel (also called "brown" channel) (Rooney et al. 2006, 2008; Zhao et al. 2018). The producer path is characterized by its relatively low diversity, low number of interactions, high cycling speed, and strong interactions; while the detritus path is characterized by the opposite (Rooney and McCann 2012). These pathways are asymmetric in the amount of energy produced by basal resources (annual production) and in the exchange rates (production:biomass ratios) (Rooney et al. 2006; Cordone et al. 2020). Consistently, theoretical and empirical approaches identify the coupling of different energy pathways by predators of large body size at high trophic positions, as a pattern that stabilizes populations and the entire community (Rooney et al. 2006; Romanuk et al. 2006; Arim et al. 2010; Rooney and McCann 2012; Keppeler et al. 2021). Each one of the energy channels varies temporarily in biomass and richness determining differences in resource availability (Ying et al. 2020). Consequently, when the green and brown energy paths are decoupled, they are prone to present oscillations and more unstable dynamics (Rooney et al. 2006, 2008). Large body size predators, at high trophic positions, are able to feed in a large range of prey directing their consumption to most abundant resources, rapidly shifting in prey type (Beckerman et al. 2010; Valdovinos et al. 2010; Jackson et al. 2011; Heckmann et al. 2012; Rooney and McCann 2012; Potapov et al. 2019). This plasticity causes predation pressure on the magnified path to increase, also reducing predation pressure on alternative energy paths. This capacity for sequential top-down pressure change would stabilize and maintain the trophic web diversity (Rooney et al. 2006; Rooney and McCann 2012). Large body size top predators, because of their highest consumption rates, relaxation in gape limitation, and greater movement capacity, mostly perform this functional role (Brose et al. 2006; Rooney et al. 2008; Arim et al. 2010; Ying et al. 2020).

One of the major global change drivers is the invasion of alien species, which generate new nodes and interactions in native communities, affecting its trophic webs by various mechanisms (Bruno et al. 2005; Rodriguez 2006; Strong and Leroux 2014; Gallardo et al. 2016). The effect of novel exotic species to the network does not necessarily imply the extinction of native species. Several studies report changes in form and function of food webs, such as changes in chain length, connectance, complexity of networks and abundances of certain trophic levels (e.g. Woodward and Hildrew 2002; Salvaterra et al. 2013; Ives et al. 2019). After the addition of an exotic species to the food web, besides the novel links with native species, there were reported changes in the interaction between native species, with shifts in their trophic habits. These shifts seem to be related to alterations in the availability and/or flow of nutrients (Strayer et al. 1999; Vander Zanden et al. 1999; Byrnes et al. 2007; Miehls et al. 2009; Pereira-Garbero et al. 2013; Strong and Leroux 2014; Ives et al. 2019). Food web complexity—e.g. richness and connectance—could dilute the invasive species impact (Carey and Wahl 2010; Garay-Narváez et al. 2013). However, community wide impacts, indirect effects, and cascade changes along the whole food web, altering their structure and functioning were frequently reported (e.g. Kimbro et al. 2009; Walsh et al. 2016; Vinagre et al. 2018; Rettig and Smith 2021). This is especially the case when the invader belongs to a new functional group or trophic level, such as a new top predator or a new herbivore (Woodward and Hildrew 2002; Gandhi and Herms 2010; Nunez et al. 2010; Thoresen et al. 2017). These invaders can produce novel ways to exploit resources and divert energy flows away from the dominant path, thus altering the whole

system stability (Miehls et al. 2009; reviewed in David et al. 2017; Vinagre et al. 2018). In spite of this, both theoretical models and empirical studies on invasions are usually limited to few species (McCann 2007).

Understanding how invaders affect the structure and functioning of trophic webs has been a central aim in invasion ecology (David et al. 2017). In several cases invaders are top predators that impact diversity at all trophic levels (Woodward and Hildrew 2002; Thomsen et al. 2014; Thoresen et al. 2017). Consequently, invasions are probably impacting the structure of coupling of energy channels by top predators, and by this mean, altering food web and ecosystem stability (Ives et al. 2019). While the coupling of energy channels is a main topic of attention in food web theory, it was rarely considered in invasion ecology, in spite of the available evidence supporting a potential impact of invaders on this mean feature of food web architecture.

The American bullfrog, *Lithobates catesbeianus* (Shaw 1802), is an aquatic amphibian categorized as one of the most dangerous invasive species worldwide (Kumschick et al. 2017; Jorgewich-Cohen et al. 2020). It is a species with a complex life cycle, so its invasion implies the addition of at least two trophospecies to native communities: post-metamorphs and larvae. Both phases present exceptionally large body sizes and commonly reach high population densities (Govindarajulu et al. 2006). While post-metamorphs are important top predators, tadpoles feed on inferior and basal resources (Ruibal and Laufer 2012; Jancowski and Orchard 2013). Regarding impacts at the community level, a decrease in abundance and richness of native amphibians has been reported (Hecnar and M'Closkey 1997a; Li et al. 2011; Gobel et al. 2019a). Contrary to what happens with most amphibian species, the bullfrog has the ability to coexist with fish. Fish presence is a strong determinant of pond community structure, limiting the richness of invertebrates and amphibians (Scheffer et al. 2006; Semlitsch et al. 2015). The evidence indicates that the bullfrog eludes this pattern, unlike most amphibians (Hecnar and M'Closkey 1997b; Babbitt et al. 2003). Whatsmore, several works show positive relationships with fishes —both native and exotic—(Maezono and Miyashita 2003; Adams et al. 2003; Laufer et al. 2008; Gobel et al. 2019a). Invaded communities show higher fish densities and lower native tadpole densities, as well as an increase in the sizes of tadpoles and fish. These changes in the community structure patterns, with alterations in the biomass of some components, suggest an effect on the relative structure of the energy paths (Maezono and Miyashita 2003; Laufer et al. 2008; Gobel et al. 2019a).

In this article, we advance on the potential effects of *L. catesbeianus* invasions on food web architecture. Specifically, using C and N stable isotopes in model ponds representative of the different pond community types (with and without fish, and with and without bullfrog), we analyzed the effect of bullfrog invasion on i) the food web architecture of integration of energetic paths, ii) the relative use of energy paths by different functional groups, and iii) the dependence on body size of the relative use of energy paths by organisms, as well as, the trophic positions at which energy paths are used.

Materials and methods

Study site

Aceguá (Cerro Largo Department) is a hilly area (approx. 220 m a.s.l.), at the northeastern Uruguayan border with Brazil (31°53′36″S, 54°09′26″W). Local land use is mostly extensive cattle and agriculture. This region hosts relatively high biodiversity of fauna and flora (Grattarola et al. 2020). At this locality, a feral bullfrog population at the establishment phase was registered in 2007. Later, in 2012 this population began the expansion, now invading 31 permanent artificial ponds, built for agricultural purposes (Laufer et al. 2018). Local ponds house relatively well-known biological communities, being fish and amphibians the most frequent vertebrates, and the bullfrog is the only exotic species reported. Fish assemblage is dominated by

the Characidae family (e.g. *Astyanax laiceps*, *Psalilodon eigenmanniorum*, *P. anisitsi* and *Cheirodon interruptus*), omnivorous species which consume detritus, algae and small crustacean and insect larvae. In less frequent Siluriformes (e.g. *Callichthys callichthys*) are also part of the assemblage (Gobel et al. 2019b). The amphibian assemblage consists of species with a wide distribution (e.g. *Boana pulchella*, *Pseudis minuta*) but also species with restricted distribution to this ecoregion (e.g. *Scinax uruguayus*, *S. aromothyella*) (Laufer and Gobel 2017). In the studied ponds, the most frequent tadpoles are *B. pulchella*, *Odontophrynus americanus*, *Pseudis minuta* and *Scinax* sp. (comprising *S. granulatus* and *S. squalirostris*, indeterminables based on their descriptions). These tadpoles feed mainly on primary producers and detritus, although they also consume low amounts of crustaceans and small insect larvae (Echeverría et al. 2007). Other frequent vertebrates are the aquatic turtles *Trachemys dorbigni* and *Hydromedusa tectifera*, both with generalist diet (Grattarola et al. 2020)**.**

Field sampling and laboratory analysis

We sampled two invaded and two uninvaded aquatic pond communities, at this locality, in April 2016. We selected these ponds based on the known local bullfrog distribution. The control (uninvaded) ponds were located near the invasion front, and *L. catesbeianus* presence has not been recorded to date (Laufer et al. 2018; NG & GL personal observation at 2020). Sampled ponds were selected according to fish presence (one with and other without fish, for the invaded and uninvaded category). The analyzed ponds had similar characteristics, they were permanent, with a range between 850 and 1780 m^2 areas. The pH of the sampled ponds ranged from 4.4 to 5.7 and the conductivity from 46 to 122 μS/cm. Because of limitations in the range of ponds with similar conditions but differing in the presence-absence of ponds and bullfrogs, and also, because our attention to food web architecture is extremely demanding in local communities information (see below), the consideration of several replicates for each condition was hampered. However, having monitored community structure in the area in previous research (Gobel et al. 2019a) we argue that this ponds are well representative of each type of pond in regard to its community structure (i..e ponds with fish and bullfrog, ponds without fish and bullfrogs, ponds with fish and no bullfrog and ponds with no fish and bullfrog) and thus can be used as a model to test for changes in food web architecture.

For stable isotope analysis, samples were taken from different basal sources, both autochthonous and allochthonous, and from various consumers. In each system, grass of aquatic and terrestrial origin, filamentous algae, decomposing plant matter, and cow feces were collected as the main basal resources. In addition, using a 20 mm diameter PVC cylinder, a 1 cm deep sediment sample was taken. Epiphyton was extracted by rinsing macrophytes with clean water and periphyton through stone scraping. In each pond, at least three replicates of each type of sample were taken and were immediately frozen at -20°C until later preparation for isotopic analysis.

Macroinvertebrates, amphibian larvae, fish, and turtles were collected using a seine fishing net (5 x 1 m area and 0.5 cm mesh). Two standardized tows were made in each pond, one in the direction of the largest diameter and the other of the smallest. From each collected taxon, between 5 and 20 specimens were taken for stable isotope analysis, covering the entire range of observed body sizes. For those cases where the number of specimens collected was less than 5 individuals per species, more tows and/or handnets passes were carried out, in plant areas and on the sediment, in order to obtain the adequate number of specimens for the analyzes (Jackson et al. 2011).

In addition, night sampling was carried out; at each pond a slow pace trail was performed by two expert researchers around the whole perimeter (Dodd 2010), where adult specimens of *L. catesbeianus* and the native aquatic frog *Pseudis minuta* were collected. This native species was especially considered since it shares habits with the bullfrog. It is an amphibian with a

completely aquatic life cycle, relatively persistent larvae and an adult predator (Huckembeck et al. 2012). Amphibians and fish were sacrificed with an overdose of eugenol (Underwood and Anthony 2013), following the protocol approved by the committee for ethics in animal research (CEUA-MNHN). Then, the specimens were determined using a taxonomic key (e.g. Ziegler and Maneyro 2008; Serra et al. 2014) and measured. Snout to tail peduncle length was measured in fish (Standard length STL), snout to tip length in tadpoles (TL) and snout-vent length in adult amphibians (SVL). Immediately, these specimens were dissected to extract a portion of muscle, dorsal in the case of fish, of the tail in tadpoles, and of the hind legs in adult amphibians. The turtles were measured in the field, where a skin sample was taken from their tails and then they were returned to the pond. All samples were immediately frozen at -20°C and transferred to the laboratory for conditioning.

In the laboratory, the samples were conditioned for the analysis following standardized procedures (e.g. Levin and Currin 2012). Grass, cow feces, filamentous algae, decomposing plant material, and sediment were washed with distilled water and analyzed under a stereomicroscope to extract animal remains. The periphyton and epiphyton samples were filtered with GF/C filters to remove the water. The invertebrates were measured (total body length, except for Coenagrionidae and Ephemeroptera which excluded the abdomen in the measurement), washed and the hard parts (e.g. elytra) were extracted and in the case of the larger invertebrates (e.g. Belostomatidae), the digestive system. Muscle and skin samples of the vertebrates were washed and conditioned. Finally, all samples were dried for 48 hours at 60°C and encapsulated in tin capsules (Levin and Currin 2012). Once conditioned, the samples were sent to the Center for Stable Isotopes of the University of New Mexico, where the analysis of stable isotopes was performed using a continuous-flow isotope ratio mass spectrometer. Finally, the correction proposed by Post et al. (2007, equation 3) was carried out for all animal tissue samples, to reduce noise signals in the $\delta^{13}C$ values, due to differences in the lipid composition of their tissues.

Estimation of biomass contribution and trophic position

Bayesian mixing models were used to evaluate the energy pathways contribution to different consumers (Parnell et al. 2013). In these models, the sources of primary producers and those of allochthonous were grouped for each pond (Fry 2013). The signal of epiphyton, periphyton and filamentous algae was considered as primary producers path, and cow feces, terrestrial vegetation, detritus of plant origin and sediment as detritus path (of allochthonous origin). Trophic Enrichment Factor (TEF) values taken from the literature were used for the different taxonomic groups. For macroinvertebrates, a fractionation of 2.5 ± 0.16 (mean $\pm$ SD) was considered for nitrogen, and 0.35 ± 0.25 for carbon, following the review of Caut et al. (2009). For anura larvae, a fractionation of 1.98 ± 0.17 and 1.69 ± 0.12 was used for nitrogen and carbon respectively (Schiesari et al. 2009). For fish, the fractionation for nitrogen for omnivorous fish of 3.4 ± 1.1 was considered according to the meta-analysis carried out by Bunn et al. (2013) and the carbon fractionation value of 0.4 ± 1.3 proposed by Post (2002). Finally, for bullfrog post-metamorphs and turtles, a nitrogen fraction of 2.3 ± 0.18 and a carbon fractionation of 1.3 ± 0.3 was considered according to McCutchan et al. (2003). We performed this analysis at the population level using the function "siarmcmcdirichletv4" and at the individual level using "siarsolomcmcv4" using the SIAR package (Parnell et al. 2013). Each model was run with 500,000 iterations and a burn-in of 50,000 times.

The trophic position of consumers was estimated, following the standard formula (Vander Zanden et al. 1997; Post 2002):

$$Trophic\ position = [(\delta^{15}N_{consumer} - \delta^{15}N_{base}) / TEF] + 1$$

where TEF is the nitrogen fractionation value and 1 represents the theoretical trophic level of the primary producers. The mean between the autochthonous and allochthonous basal sources of each pond was used to estimate the trophic position. Specific TEFs were used for each taxonomic group, corresponding to those used in the mixing models (see above).

Statistical analysis

The relationship between the maximum trophic position ($\sim\delta^{15}N$) and the balance in energy sources ($\sim\delta^{13}C$) used by species was analyzed by quantile regressions (Cade and Noon 2003). The variable $\delta^{13}C$ was centered to standardize variations in the different communities analyzed. The effect of native fish and the invasive bullfrogs was analyzed, as well as the interaction between these effects. Models were selected with the AIC criterion and the weight of evidence (Burnham and Anderson 2002). A stepwise model selection was used to advance the identification of plausible models.

For each community, the relative importance of each energy path in consumer biomass was evaluated. The change in invertebrate and characid fish proportion of allochthonous biomass was compared among sites with and without bullfrog, through an Analysis of Variance test. In order to consider the pond identity effect, this variable was included into the model as a factor. For these analyses, we used each individual value of proportion of allochthonous biomass as a replicate. Native tadpoles were excluded because of their low frequencies in the invaded ponds. Then, we performed a linear model to explore the effect of individual body size on the proportion of allochthonous carbon assimilated and trophic position. For each assemblage (characid fish, native tadpoles and macroinvertebrates), we perform independent models, using least square regression. The individual proportions of allochthonous biomass and trophic position was considered as the dependent variable; body size and bullfrog presence (and its interaction) were the independent variable. Native tadpole body sizes were log transformed because of their nonlinear association with trophic position and energy sources and to satisfy model assumptions of normality and homoscedasticity. Furthermore, the proportion of allochthonous carbon assimilated and trophic position in relation to body size was evaluated for both post-metamorphs and larvae of *L. catesbeianus* by linear models. Model selection was made by the Likelihood ratio test (LRT) (Zuur et al. 2007). In all cases, we considered $\alpha = 0.05$ as statistically significant and the analyzes were performed in the R software (R Core Team 2021).

Results

Integration of energetic paths by species at higher trophic positions was a preponderant pattern in all the analyzed pond communities, independently of the presence of fishes or bullfrogs (Figure 1). The 99th percentile of individual $\delta15N$ presented a cubic relationship with $\delta13C$ and a significant interaction with the presence of fish and bullfrog in the pond. However, it has to be highlighted that the qualitative pattern was the same for all the analyzed communities, independent of the predators taxonomic identity (fish, amphibians and/or turtles), following an inverse U-shaped trend in which species at higher trophic positions have a balanced use of energy sources (Figure 1, Online Resource 1).

Macroinvertebrates and Characidae fish assemblages showed significant differences in the assimilation of allochthonous carbon into their biomass in the bullfrog invaded and uninvaded systems (Figure 2). While the macroinvertebrates showed a lower proportion of allochthonous carbon assimilated into their biomass in the invaded systems ($F_{1,50} = 20.0$, $p < 0.001$), fishes exhibited a higher proportion ($F_{1,56} = 135.0$, $p < 0.001$). The pattern of fish assemblage increase in the proportion of allochthonous supported biomass was also observed at species level (*A. laticeps*, *P. anisitsi* and *C. interruptus*), with the

Bayesian mixing models at the taxonomic group level. This analysis also showed low allochthonous supported biomass for most of the invertebrate taxa in the invaded ponds (Online Resource 2).

Body sizes presented a significant association with the fraction of biomass assimilated from allochthonous sources along all the taxa considered. Macroinvertebrates assimilation of allochthonous sources increased with body size in all systems ($F_{1,49} = 9.27$; $P = 0.004$) but having a significantly higher proportion in uninvaded ponds ($F_{1,49} = 23.3$; $P < 0.001$). The main difference observed between invaded and uninvaded ponds was a shift in the fraction of allochthonous biomass, and not a change in taxonomic identity (Jaccard similarity between communities = 0.75). The macroinvertebrates of the invaded ponds had a fraction of allochthonous consumption 15% lower than in the uninvaded ones. Likewise, in all ponds, the larger individuals presented 15% more of their biomass being subsidized from the allochthonous path than the smaller body sizes (Figure 3a). Native tadpoles also exhibited an increase in the fraction of allochthonous biomass assimilated in their biomass with the logarithm of body size, going from 50 to 60%, between small and large sizes ($F_{1,10} = 19.2$; $P = 0.001$), without significant differences between invaded and uninvaded ponds ($F_{1,10} = 0.03$; $P = 0.9$; Figure 3b).

Fish did not change the proportion of allochthonous assimilation in their biomass with body size in the uninvaded pond, as happened in the invaded system. Conversely, fish at the invaded pond significantly increased their allochthonous supported biomass along their body size range. Fish that coexisted with bullfrogs presented a proportion of 50% allochthonous supported biomass in the smallest sizes and a proportion of 75% in the largest sizes, while fish in the control site (uninvaded pond) presented a contribution from this source of approximately 30%, independently of individual body size (Figure 3c). In the selected model, the interaction between body size and bullfrog presence was statistically significant ($F1,54 = 38.7$, $P < 0.001$).

Macroinvertebrates, amphibians and fish trophic position exhibited a significant association with body sizes (Figure 3). Macroinvertebrates decreased trophic position with body size ($F_{1,46} = 7.9$; $P = 0.007$), being on average 0.7 higher in invaded ponds ($F_{1,46} = 12.5$; $P < 0.001$; Figure 3d). Native tadpoles also decreased trophic position with log of body size ($F_{1,9} = 37.4$; $P < 0.001$; Figure 3e), unchanged between invaded and uninvaded systems ($F_{1,9} = 3.0$; $P = 0.1$). On the contrary, fish evidenced an increase in the trophic position with body size ($F_{1,54} = 21.7$; $P < 0.001$), being significantly higher in the site not invaded by bullfrog, with an average difference of 0.3 ($F_{1,54} = 32.0$; $P < 0.001$; Figure 3f).

Post-metamorphic *L. catesbeianus* exhibited a significant association between the fraction of allochthonous supported biomass and body size, but a relatively low explained variance ($F_{1,18} = 7.7$, $P = 0.01$; r-sqrt = 0.26; Figure 4a). Bullfrog tadpoles showed a positive association between body size and the proportion of allochthonous biomass ($F_{1,12} = 23.3$, $P < 0.001$; r-sqrt = 0.63). During this developmental stage, the fraction of biomass from allochthonous sources ranged from 40 to 70%, increasing along the observed body size gradient (Figure 4b). Bullfrog individual trophic position did not show a relationship to body size, neither for the post-metamorphs ($F_{1,18} = 0.5$; $P = 0.5$), nor for their larvae ($F_{1,12} = 0.004$; $P = 0.9$). The post-metamorphs showed a trophic position that varied between 2.5 and 4, while the larvae varied between 2.3 and 2.6 (Figure 4c, 4d).

Discussion

The consequences of biological invasions on biodiversity patterns and stability are mediated by changes in food web architecture (McCann 2007), however, the study of these changes was underrepresented in the invasion literature (Simberloff 2004). In this research we analyzed the impact of invasions in key structural features of food webs. We observed both, invariant and altered

components of food web organization putatively determined by bullfrog invasion. On one hand, despite the invasion and strong changes in community organization, the integration of energetic paths by large predators at upper trophic positions remains as a pervasive feature of food webs (Figure 1). This integration was repeatedly identified as a main determinant of ecosystem stability (Rooney et al. 2006; Arim et al. 2010; McCann 2011; Rooney and McCann 2012). On the other hand, the relative use of energy channels by different functional groups, as well as its dependence on body sizes, was significantly impacted by bullfrog invasion.

Trophic web structure, associated with the integration of energy pathways at higher trophic positions, was remarkably robust to *L. catesbeianus* invasion. These exotic frog post-metamorphs would operate as top consumers in upper trophic positions, integrating different energy pathways, a functional role performed by native predators (fish, turtles and the aquatic frog *P. minuta*) among non-invaded communities. Paths integration at high trophic positions implies that consumers progressively add new energy sources by increasing their position in the food web (Rooney et al. 2006; Arim et al. 2010; Rooney and McCann 2012). This is a consequence of both, the increase in its consumption capacities (in terms of the prey access), as well as greater energy demands related to body size (McCann et al. 2005; Arim et al. 2007). Thus, predators at higher trophic positions within the food web would present a progressively more balanced consumption of the different available sources. The robustness of this pattern, despite top predators identity and community assembly is consistent with its proposed role in ecosystems (Rooney et al. 2006), coupling of local communities (McCann et al. 2005), the fulfilling of energetic demands (Arim et al. 2010, 2016), and the integration of fast—green—and strong—brown—pathways stabilizing food webs (Rooney and McCann 2012; Segura et al. 2017). This suggests that, despite the impact on the invaded community with changes in species composition, body size and biomass–such as was reported for vertebrates at the studied site (Gobel et al. 2019a)–this main feature of food webs architecture is preserved. The introduction of top predators has been associated with changes in the upper trophic level and in other components of the web (Vander Zanden et al. 1999; Baxter et al. 2004). However, its effect on energy pathway integration patterns had not been considered. The identification of structural aspects that are robust or vulnerable to invasion processes is key, both for understanding the mechanisms behind the impact of invasions, and for understanding the trophic web in general.

Not all features of biodiversity organization are equally sensible to invasion effects. In this sense, having reported the impact of invasions on different components of biodiversity (Lockwood et al. 2006), there is a need for the evaluation of those attributes that remain invariant after invasions. Particularly, to those properties that if disrupted—e.g. decoupling of energy channels—may determine an abrupt change in communities stability. Biodiversity architecture has shown some invariant organization despite changes in environment, human impact and taxonomic composition (e.g. Marquet et al. 1995). Invariant attributes may be associated with tipping points in ecosystems resistance/stability (Tylianakis et al. 2010; Rooney and McCann 2012). In this sense, an important aspect is to understand what happens if the impact of the invasions is great enough to change these invariant characteristics. The bullfrog invasion in Aceguá is an interesting model to this aim. It is located in a relatively conserved area, with a unique and recent invasive top predator. Until now, these systems were strongly impacted by the bullfrog invasion changing diversity and biomass along all trophic levels (Laufer and Gobel 2017; Gobel et al. 2019a), but not affecting the integration of energy channels by large predators at upper trophic positions. It should be noted that in spite of these changes, community diversities and biomass along different functional groups remain high (Gobel et al. 2019a). However, further habitat degradation and climate change may surpass communities capacity to preserve this feature of food webs, surpassing a tipping point after which the whole system is strongly degraded (Jackson et al. 2017).

Both, diet studies (e.g. Jancowski and Orchard 2013), as well as the insertion pattern in the trophic network, confirm that bullfrog is a generalist predator with high plasticity and an exceptional capacity to exploit local resources. Evidence from the study site

and elsewhere show that bullfrog is able to feed on practically any prey it can ingest, from small insects to vertebrates, or even conspecific (Laufer et al. 2021). Surely, this ability to integrate different trophic pathways sustains these populations of large body sized predators, and therefore with high-energy requirements. In fact, in our studied systems the isotopic signal observations evidenced that adult bullfrog integrated between 39 and 70% of carbon from the debris pathway. In spite of the several studies analyzing bullfrog diet no attention was previously devoted to these features. The insertion patterns of individuals in the food web exhibit a strong dependence on body size, with variations in their trophic position and in the consumption of different energy pathways (Woodward et al. 2005; Arim et al. 2010; Jardine et al. 2017).

Bullfrog invasion also involves a persistent, big sized tadpole with a body size and life history strategy that strongly differ from native amphibian biology (Govindarajulu et al. 2006; Vera Candioti 2007; Schiesari et al. 2009). Bullfrog tadpoles were identified as intermediate omnivores, in a similar trophic position to predatory insects and only below the predators with upper trophic positions in the system. These larvae body size is twice the largest of the native tadpoles and also reaches high densities. It has been recognized that many anuran larvae act as predators and not only as herbivorous (Altig 2007). In this sense, although bullfrog tadpoles present a generalized anatomical oral disc configuration (Altig and Johnston 1989), their large body size and the consequent release of size restrictions on the prey consumption, suggests that they could act as consumers. Previous studies of diet and stable isotopes indicate that these tadpoles may be preying on eggs and early stages of different species (Schiesari et al. 2009; Ruibal and Laufer 2012). Congruently, the present results place them in high trophic positions, not mainly as basal consumers. Further, our results also support that the increase in energy demand with body size is probably attended by an increase in the proportion of detritus or detritivores that are consumed, but having no change in trophic position—probably because their morphology forbids the consumption of large prey at higher trophic positions. In fact, almost 60% of the biomass of the largest bullfrog larvae (more than 90 mm) came from the detritus path, while this value does not reach 40% in the smallest individuals. Possibly, at low body sizes, these larvae assimilate a greater fraction of energy from the green path, which has a higher nutritional quality, adding then other resources to sustain their requirements (Jardine et al. 2017).

A point to highlight is the contrasting trend observed in fish and macroinvertebrate species among invaded and non-invaded communities. At this point we must take into account that in the observed communities a strong loss in the abundances of native tadpoles has been reported, due to the invasion of the bullfrog (Gobel et al. 2019a). Both persistent assemblages, fish and macroinvertebrate species, changed their energy sources balances but in different ways. Fish species increased the proportion of allochthonous supported biomass in invaded ponds but the opposite was true for macroinvertebrates. Turnover in species composition may explain these trends but we did not observe a change in invertebrate species composition, suggesting a change in species diet and their associated functional roles. The variability and plasticity in the consumption of different paths between individuals of the same species can result in an adaptive capacity, with unknown effects on the stability of the trophic webs (Elliott Smith et al. 2021). Furthermore, macroinvertebrates have the ability to alter their consumption based on the availability of resources (Entrekin et al. 2020). Possibly in invaded ponds macroinvertebrates shift towards greater autochthonous assimilation due either to a lower use of this resource by vertebrates, a displacement of invertebrates from allochthonous resources because or their use by vertebrates, predation avoidance behaviour or direct interference with vertebrates consuming allochthonous resources. In this sense we stress that given that most algae on these systems grow attached to macrophytes, a displacement of macroinvertebrates from the bottom to macrophyte patches-perhaps seeking refuge from predation or from bioturbation- may promote a diet including more algal resources (Hansson et al. 2010).

The three most abundant fish species (*A. laticeps*, *P. anisitsi* and *C. interruptus*), had a high assimilation of detritus (Online Resource 2). Local diet studies report an omnivorous consumption for these three species (Gobel et al. 2019b), so we assume

that their decrease in trophic position probably was associated with a greater availability of basal resources in the allochthonous path. In fact, the pattern that we have identified for these fish in invaded waterbodies was an increase in the abundance of larger sizes (Laufer et al. 2008; Gobel et al. 2019a). Bullfrog presence in a pond could generate an availability of resources associated with this path. This release of resources by invasive species is a frequent phenomenon, affecting environmental conditions and ecosystem functioning (reviewed by Gallardo et al. 2016). This type of positive interaction between invasive and native species has been scarcely explored, representing a priority for understanding the whole range of mechanisms impacted by biological invasions (Gallien and Carboni 2017). The pattern reported here supports the idea that the access to more resources from allochthonous paths in larger fish sustains their densities increase. However, the proximate mechanisms involved are not evident. Bullfrog larvae also increased the assimilation of the detritus path with increasing body size (Figure 3 and 4). This shift could be related to a change in trophic habits, and/or to changes in assimilation capacity, during larval ontogeny (Smith 1999; Jardine et al. 2017). Both fish and bullfrog larvae had similar biomass fraction from the detritus via (Online Resource 2). This suggests that the support of large bullfrog tadpoles and fish populations would be related to a very similar trophic pathway. Considering the long period of persistence of the bullfrog larval stage (Govindarajulu et al. 2006), and their high densities at the studied systems, their high mobility could generate significant pond bioturbation (Ranvestel et al. 2004). In this way we can hypothesize two possible mechanisms in which bullfrog larvae amplify their use of the debris trophic pathway, also favoring its use by fish. First, bullfrog larvae through bioturbation and/or consumption and excretion could amplify the detritus path, making it available to other community components. Furthermore, this increase in suspended sediment in the water column would decrease light penetration, and therefore restricts the primary producer's path. This negative effect on the producers would have important implications on the function of the food webs (Thorp and Delong 2002; Marcarelli et al. 2009). This benthos disturbance would reduce shelters and crypsis to local detritivorous invertebrates fostering their consumption by fish and bullfrogs. Secondly, another possible mechanism would be the transformation of detritus into an important biomass of bullfrog larvae, whose skin or secretions could be consumed by fish. Future studies of stable isotopes with a greater number of evaluated communities, as well as lipid profile studies (Whiles et al. 2010) of both species and mesocosm experiments (Dodd 2010) could shed light on these hypotheses.

Most of the changes observed in the invaded trophic webs were dependent on body size. This is a key attribute in the food web structure and function, and many traits scale with it, such as energetic demand, vulnerability and capacities/potential for trophic interactions, speed and potential distance of movements (Brown et al. 2004). Consequently, it's widely recognized that mass-dependence of the mechanisms determining food web structure and functions (Brown et al. 2004; Sibly et al. 2012). Not surprisingly, understanding the impact of invasions on food webs may be also improved by taking a size-dependent perspective. This work involved a compromise between the trophic and taxonomic resolution of the communities and the number of systems that can be analyzed at this level. Thus, we must be cautious in the interpretation of certain results, considering that there are other ecosystem factors of the ponds (e.g. area, pH, vegetation cover and impact of cattle, among others) that could also explain the observed changes (Schalk et al. 2017). The focus of this work on the structure and function of the trophic web aims to contribute to aspects of invasion theory where there is scarce available knowledge. This decision has the cost of the limits of all natural experiments where the causal direction can be opposite to the process considered (Shipley 2016). Recognizing these limitations, this contribution is one of the first pieces of evidence about the effect of the bullfrog invasion on the structure and functioning of energy pathways in native trophic networks.

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Statements and Declarations

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Figure Caption

Fig 1 Trophic position and energy sources in ponds with and without fishes and with and without bullfrogs. The $\delta 15N$ is presented as a function of the centered $\delta 13C$. The weight of the selected model was close to 1 (wi~1). In spite of turnover in the upper predator identity among ponds, in all the systems as the organisms are observed at higher trophic positions energy sources become constrained to a simultaneous use of the brown and green energy paths

Fig 2 Proportion of biomass from allochthonous sources for macroinvertebrates ($F_{1,50} = 20.0$, $p < 0.001$) and fishes ($F_{1,56} = 135.0$, $p < 0.001$) in communities invaded and uninvaded by bullfrogs

Fig 3 Proportion of allochthonous supported biomass and trophic position in relation to body size for native assemblages: macroinvertebrates (Proportion of allochthonous supported biomass: $F_{2,49} = 16.3$; $P < 0.001$; r-sqrt = 0.37. Trophic position: $F_{3,46} = 6.8$, $P < 0.001$; r-sqrt = 0.26), native tadpoles (Proportion of allochthonous biomass: $F_{2,10} = 9.6$; $P = 0.004$; r-sqrt = 0.59. Trophic position: $F_{3,9} = 13.2$, $P > 0.001$; r-sqrt = 0.70) and Characidae fishes (Proportion of allochthonous supported biomass: $F_{3,54} = 137$, $P < 0.001$; r-sqrt = 0.88. Trophic position: $F_{3,54} = 18.9$, $P < 0.001$; r-sqrt = 0.48) in communities invaded (red) and uninvaded (blue) by bullfrogs. In all graphs the regressions predicted by the models are shown. If there are no differences between invaded and non-invaded sites, it is shown as a black line

Fig 4 Fraction of allochthonous supported biomass and trophic position in relation to body size for post-metamorphs (Proportion of allochthonous biomass: $F_{1,18} = 7.7$, $P = 0.01$; r-sqrt = 0.26. Trophic position: $F_{1,18} = 0.5$; $P = 0.5$, r-sqrt = -0.03) and tadpoles (Proportion of allochthonous biomass: $F_{1,12} = 23.3$, $P < 0.001$; r-sqrt = 0.63. Trophic position: $F_{1,12} = 0.004$; $P = 0.9$; r-sqrt = -0.08) of the invasive bullfrog *Lithobates catesbeianus*