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American bullfrog (*Lithobates catesbeianus*) distribution, impact on native amphibians and management priorities in San Carlos, Uruguay

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Abstract – Biological invasions are a major cause of biodiversity and ecosystem services loss. However, information on distribution and impacts is limited for many alien species, restricting the development of local management measures. The aim of this study is: to identify the current situation of the American bullfrog (*Lithobates catesbeianus*) invasion focus in San Carlos (Maldonado, Uruguay); to evaluate its impacts on native anurans; and to provide management tools. Between 2017 and 2019, 75 permanent ponds were sampled, finding an expanding bullfrog population (occupying 32 ponds, in 16.5 km^2). Results show that native anuran richness was lower in the invaded ponds. Observed impacts were greater for the aquatic frog *Pseudis minuta*, probably due to greater encounter rates with the invader. The abundance of tadpoles was also lower in the invaded ponds. The local pond network was explored using graph theory, evaluating its topological role and centrality. In this network, a list of priority ponds was generated to prevent local bullfrog expansion. Given the relatively small size of this population, eradication seems feasible. Focusing on the key nodes could prevent further expansion, by using spatial prioritization to organize the recommended management of the pond network.

Keywords: Community structure / risk map / invasive species / aquatic frog / *Rana catesbeiana*

1 Introduction

Biological invasions are a major component of global change and are expected to maintain their growth rate in the near future (Seebens *et al*., 2017). Human activities move an increasing number of species outside their historical distribution ranges, and some of them manage to get established and invade new regions. These invasions can affect ecosystem services and decrease native species abundance and richness through mechanisms such as predation, competition, indirect interactions, disease transmission and hybridization (Ricciardi *et al*., 2017). Although important advances have been made in the last decade concerning how invasions influence the conservation of biodiversity, economy and human health, there is still a great lack of knowledge about the state and the impacts of many invasive species worldwide (Latombe *et al*., 2016). This lack of information is even greater in some regions, such as South America, where studies are comparatively scarce, and usually report only occurrence or anecdotal data of invasive alien species (Speziale *et al*., 2012; Ballari *et al*., 2016; Schwindt and Bortolus, 2017).

The American bullfrog, *Lithobates catesbeianus* (Shaw 1802) is an invasive species of concern, as it is expanding its global range. This species, native to eastern North America, has been introduced into several regions, mostly to be cultured for human consumption (Kraus, 2009). The bullfrog is a large-bodied aquatic anuran with great ecological plasticity. It has the ability to exploit diverse resources, and to tolerate a wide range of environmental conditions. This invasive species shows a preference to occupy permanent lentic systems (Adams and Pearl, 2007), commonly named ponds, where it inhabits throughout its ontogenic cycle (Minowa *et al*., 2008; Cook *et al*., 2013). Bullfrog juveniles and adults have the ability to disperse between these systems on land, and thus expand its distribution. The availability of these ponds determines bullfrog colonization and expansion in the landscape (Blaustein and Kiesecker, 2002; Ficetola *et al*., 2010). Thus, a bullfrog invasion is a spatially structured population that can be seen as a network of nodes (ponds, discrete patch habitats) interconnected by its dispersal capacity in a suboptimal environment (Descamps and De Vocht, 2016).

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For this reason, the graph theory approach can be an interesting management tool for quantifying connectivity between ponds and exploring the bullfrog invasion risk (Drake *et al.*, 2017).

Adult bullfrogs are important predators and strong competitors for food resources (Kiesecker *et al.*, 2001; Jancowski and Orchard, 2013; Laufer *et al.*, 2021). Tadpoles are also predators and habitat disruptors by bioturbation (Kupferberg, 1997; Kiesecker *et al.*, 2001; Ruibal and Laufer, 2012; Gobel *et al.*, 2019). Although we know that bullfrog invasion can alter different components of invaded communities (*e.g.* Gobel *et al.*, 2023), we know little about its effects on different taxonomic groups (mostly from diet records; Jancowski and Orchard, 2013). Most of the studies have focused on its impacts on native amphibians. In addition, *L. catesbeianus* has recently been identified as a vector and reservoir of global amphibian diseases, especially Ranavirus and Chytridiomycosis (Garner *et al.*, 2006; Lesbarrères *et al.*, 2012; Schloegel *et al.*, 2012; Ribeiro *et al.*, 2019).

The spread of this invasive frog was related to local changes in the structure of amphibian assemblages and their acoustic niches, and population declines (Fisher and Shaffer, 1996; Hecnar and M'Closkey, 1997; Li *et al.*, 2011; Both and Grant, 2012; Liu *et al.*, 2017). Evidence suggests that large sized bullfrog adults may behave as important predators of amphibians (Liu *et al.*, 2018; Bissattini *et al.*, 2018; Oda *et al.*, 2019; Laufer *et al.*, 2017, 2021). In addition, bullfrog juveniles can also be strong competitors of native amphibians (Silva *et al.*, 2016). In fact, Bissattini and collaborators (2019) found a shift from competitor to predator behavior, during bullfrog ontogeny. Kats and Ferrer (2003) reviewed bullfrogs' negative effects in Western North America, and predicted that its global spread would impact amphibians in new regions.

The bullfrog is becoming a frequent invader in the Neotropical region, with several foci recently reported in Argentina, Brazil, Venezuela, Ecuador, Colombia, and Uruguay (Barbosa *et al.*, 2017). Therefore, it is important to understand its effects on native amphibian assemblages, as well as which species would be most affected, to generate control strategies and mitigation practices (Adams and Pearl, 2007). In this sense, the literature suggests that the most affected species would be those that share microhabitat use with bullfrogs (Pearl *et al.*, 2004). In this context, it is important to explore the effects of this invasion on native amphibian assemblages in permanent water bodies and to assess which aquatic species are being most affected.

The bullfrog was introduced in Uruguay in the 1980 s at 19 aquaculture farms for producing frog legs for human consumption, a project which did not become a profitable operation and collapsed in the early 2000 s. This industry was not strictly controlled by national authorities, and the environmental risks involved were underestimated. Thus, invasion foci emerged in some of the sites where bullfrog farms were established (Laufer *et al.*, 2008, 2018a, 2018b). One of these farms, Laguna Dorada, was located in Southeastern Uruguay, in San Carlos (Maldonado Department), and was one of the largest bullfrogs farms from 1993 to 2001. In 2007, as part of a national survey of old bullfrog farm areas, a search for bullfrogs in San Carlos was conducted and no feral individuals were detected (Laufer *et al.*, 2018a). Later, in 2015, the presence of feral bullfrogs was reported in a pond located over a kilometer away from the Laguna Dorada farm facilities (Lombardo *et al.*, 2016). A few months later, six additional invaded water bodies were reported, thus confirming that the first pond represented a larger population. Emphasis was placed on further efforts to evaluate the bullfrogs' real distribution and ecological impacts in the area (Laufer *et al.*, 2018a).

Fig. 1. Photographs of two ponds sampled in San Carlos, Maldonado Department (Uruguay), showing the differences in macrophyte cover.

The goal of this study was to evaluate the current invasion degree, and its effects on the native amphibians in San Carlos. In addition, management tools were to be generated from the understanding of the local network of environments susceptible to be invaded by bullfrogs. As a working hypothesis, it was proposed that changes in San Carlos amphibian assemblages' structure are explained by bullfrogs preying on and competing with species of this group. Therefore, it is expected to find lower richness and abundance of native amphibians in ponds invaded by bullfrogs. This effect should be stronger for those native anurans which most interact with bullfrogs, due to their similar microhabitat use.

2 Materials and methods

2.1 Study site

San Carlos is located in Southeast Uruguay in the Merín Lagoon Graven ecoregion, 15 km from the Atlantic Ocean (Brazeiro, 2015). It is a plain area (average height 20 m a.s.l.), where San Carlos Stream flows into the Maldonado Stream. The region has a national conservation priority due to its unique assemblages and high biodiversity (Di Minin *et al.*, 2017; Grattarola *et al.*, 2020). It is a peri-urban area, with a mosaic landscape of agricultural activities, which means that there is a great density of permanent water bodies, frequently used as reservoirs for farms (Álvarez *et al.*, 2015). Those water bodies, called ponds further on, were characterized as having a pH of 7.24 ± 1.06 (mean ± SD), a conductivity of $203.09 \pm 212.70\ \mu S \cdot m^{-1}$, an area of $902 \pm 1262\ m^2$, $46.0 \pm 44.0\%$ of macrophyte coverage, and fish in 54% of them (unpublished data, based on 26 sampled ponds during the spring of 2017). These ponds (Fig. 1) are usually inhabited by native anurans, which use them for breeding and foraging (Arrieta *et al.*, 2013; Grattarola *et al.*, 2020).

2.2 Post-metamorphs: sampling, mapping and analysis

Four surveys were completed during the spring and the summer: 8–10 December, 2017; 19–21 October, 2018; 20 March, 2019; and 6–8 December, 2019. We sampled all the ponds peripheral to those where we knew the bullfrog was reported in previous studies (Lombardo *et al.*, 2016; Laufer *et al.*, 2018a). The limit of the sampling area was established by a buffer around the invaded zone (where bullfrogs were not detected). We then complemented this limit with visits to peripheral areas during periods of bullfrog vocalization, confirming that there were no other foci in the area. This sampling area allowed us to cover 75 ponds, invaded and not invaded by bullfrog, within the considered area. These ponds were standardly sampled once during these campaigns (in total by the end of the surveys, we visited once each pond), between 8:00 p.m. and 12:00 a.m. that is within the period of highest bullfrog (Laufer *et al.*, 2017) and native anuran activity (Moreira *et al.*, 2007). At each pond, a slow walk was taken by two researchers around the whole perimeter, for a minimum of five and a maximum of ten minutes, depending on the pond size (Dodd, 2010; Heyer *et al.*, 2014). The total number of post-metamorphic (*i.e.* juveniles and adults) amphibians observed were recorded for each species (direct count of bullfrog and native anurans). Bullfrog calling activity was categorized as: 0–no records; 1–a single individual vocalizing; 2–two or three individuals vocalizing; 3–more than three individuals vocalizing, and still being able to individualize the calls; and 4–chorus, where calling individuals were not identifiable (Dodd, 2010). In each of the campaigns, both types of ponds were sampled, (invaded and uninvaded by bullfrogs) and environmental variables at that specific time were recorded. These variables, along with the sampling date, are useful for standardizing and understanding differences between campaigns. During sampling, air temperature, humidity, wind intensity and percent of cloud coverage were registered.

The 75 sampled ponds were classified into four categories, according to the bullfrog field data collected: 0–no records; 1–one individual observed and/or heard; 2–between 2 and 5 individuals observed and/or heard in calling category 2 or 3; and 3–more than 5 individuals observed and/or heard in calling category 4 (chorus) (Heyer *et al.*, 2014). Bullfrog distribution was mapped using ArcGIS Pro software (ESRI, 2021), and ponds were allocated to the above-mentioned categories. The distribution areas were obtained from the polygons generated by the union of the external ponds, and adding a buffer area of 727 meters (considering the maximum terrestrial dispersion distance of bullfrog in a similar environment in Belgium; Descamps and De Vocht, 2016).

Richness (total number of species observed or heard) and abundance (total number of individuals observed) of the native anuran assemblages were compared between invaded and uninvaded ponds. Invaded ponds were considered those with any bullfrog record, regardless of density. For this analysis, 60 comparable ponds (for which data was complete) were considered, 27 of which were invaded by bullfrogs. Post-metamorphic anuran richness (number of species observed or heard) and abundance (direct counts from visual observations) were tested to determine they were related to the presence-absence of bullfrogs by fitting a generalized linear model (GLM), with a negative binomial distribution. This distribution is commonly used to count variables with overdispersion. Air temperature, humidity, wind intensity and percent cloud coverage were tested as possible explanatory variables in the model. Model selection was done using the Likelihood Ratio Test (LRT). Sampling date was always included as an explanatory variable in the model, in order to consider the variation introduced by the sampling design. Finally, a residual analysis was performed to check the homoscedasticity and normality of each model's residuals (Zuur *et al.*, 2007).

The specific abundances (direct counts from visual observations) of the three most-frequent native species were explored (*i.e.* post-metamorphic individuals recorded in more than 15 ponds): *Boana pulchella*, *Leptodactylus luctator* and *Pseudis minuta*. These three species differ in many ecological traits. *Boana pulchella* is a mid-sized arboreal species, inhabiting peripheral trees and shrubs, using the ponds' macrophytes for calling and reproduction. *Leptodactylus luctator* is a big sized cursorial species, which reproduces in ponds and displays parental care. *Pseudis minuta* is a fully aquatic mid-sized species, inhabiting the pond during its entire ontogeny. This frog commonly swims and floats on the surface (Melchiors *et al.*, 2004; Moreira *et al.*, 2007). For these three species, a negative binomial GLM was used to test the association between post-metamorph abundance and observed bullfrog abundance following the above-mentioned procedure (Zuur *et al.*, 2007). In this case, the sampling date was used as an explanatory variable, in order to consider the variation introduced by the sampling design.

2.3 Tadpole: sampling and analysis

From the 8th to 10th of December, 2017 a subset of 26 ponds (19 of them invaded by bullfrogs) were sampled diurnally with hand nets to study tadpole assemblages (Dodd, 2010; Heyer *et al.*, 2014). These ponds were selected because they were located within the center of the invasion and because they presented similar and comparable conditions. Pond area was determined using a hand-GPS. Tadpole sampling was done at five equally distant stations and covering different environments: three in the vegetated shallow areas and two in the deep areas. At each station, three standardized hand net passes of 2 m each were performed. The identity and number of tadpoles collected were recorded and then released. The percent of coverage by emerging and floating macrophytes was visually estimated. The presence of fish was considered as a binomial variable, according to the presence-absence of any fish in the hand net samples. The most frequent fish were Characidae and Poeciliidae. Fishes reach significant abundances in these ponds and have a generalist diet, thus excluding many amphibian species (Teixeira de Mello *et al.*, 2011). This variable was recorded due to the strong available evidence showing that fish presence determines the structure of the amphibian community (Hecnar and M'Closkey, 1997).

Species richness and abundance of the native anuran tadpole assemblages between invaded and uninvaded ponds were compared. Invaded ponds were those considered as having any bullfrog record, regardless of density. A negative binomial GLM was used to test the association between tadpole richness and abundance and the presence-absence of bullfrogs following the above-mentioned procedure. Pond area, fish presence, and percentage of macrophyte cover were tested as possible explanatory variables in the model. The best models were selected using the LRT (Zuur *et al.*, 2007). All data analyses were performed in R open software, with

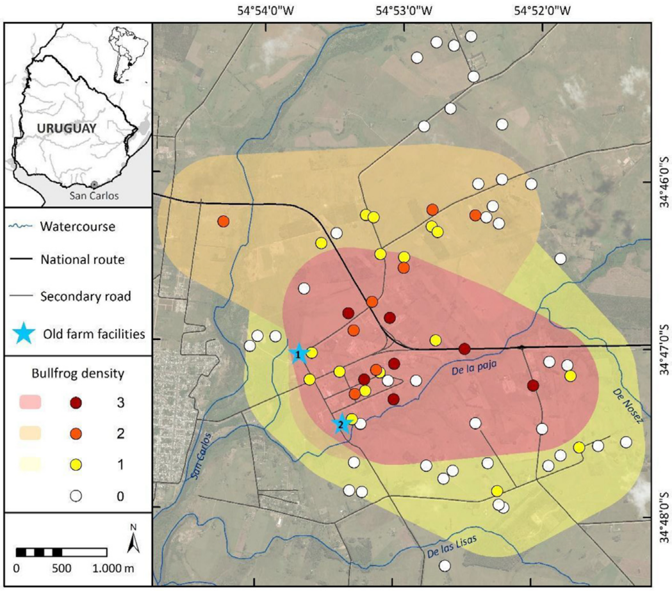

Fig. 2. Distribution area of *Lithobates catesbeianus* in San Carlos, Maldonado Department (Uruguay). Estimated bullfrog density levels are shown: 0 in white, 1 in yellow, 2 in orange, 3 in dark red. Location of the old farms facilities (sky-blue stars): 1, registered farm (Laufer *et al.*, 2018a), 2, unregistered farm. Circles: sample ponds, shaded areas encompass polygons of invaded ponds and a 727 m buffer area.

$\alpha = 0.05$ as the criterion for statistical significance (R Core Team, 2019).

2.4 Graph analysis

Since American bullfrog dispersion consists in the occupation of discrete areas (permanent ponds), understanding and predicting its invasion process requires understanding the spatial structure and connectivity of these systems at the landscape level (Adams and Pearl, 2007). San Carlos' pond network was explored using graph theory, an approach that allows quantifying the risk of invasion based on the landscape structure, and the species' dispersal ability (Minor and Urban, 2008; Drake *et al.*, 2017). Pond locations were obtained from the hydrological shape available at the Infraestructura de Datos Espaciales del Uruguay (https://visualizador.ide.uy/ideuy/core/load_public_project/ideuy`), with a buffer of 4650 m (the greatest distance between two invaded ponds) around the bullfrog invasion area that was mapped in San Carlos. Then, the euclidean distances between the centroids of each pond was determined, and those pairs of ponds that were distanced at less than 727 m (maximum dispersal distance of *L. catesbeianus* according to Descamps and De Vocht, 2016) were considered connected. Based on this criterion, the topological pond network was constructed. This model tacitly assumed that bullfrog movement ability was homogeneous across landscape, not considering other factors that could promote its dispersion (Fletcher *et al.*, 2011; Drake *et al.*, 2017), such as human activity and surrounding environmental features (*e.g.* lotic water bodies). In any case, the bullfrog is known to preferentially inhabit ponds, where it reproduces and grows, and only occasionally uses other aquatic environments such as creeks, streams or temporary ponds (Blaustein and Kiesecker, 2002). In addition, during our repeated surveys in the area we have not recorded the presence of bullfrog in lotic systems (unpublished data). Considering that the topography of the area is predominantly low-lying, it is unlikely that the bullfrog will move mostly through the water currents to invade the ponds. In any case, we do not have standardized data for the area that allows us to evaluate this possible bias.

Based on the graph model, the ponds that would play an important role (because of their location and connectivity in the network) in the local dispersion of the bullfrog in San Carlos were selected. These would be the ponds in which management is a priority to slow down the invasion: the stepping stones, peripheral hubs and connectors. The so-called stepping stones, those ponds that allow the shortest paths through the network, were obtained by the betweenness centrality metric, using the igraph R package. For the prioritization, 5% of the ponds with the highest betweenness centrality values were selected (Newman and Girvan, 2004; Csardi and Nepusz, 2006). Then, the modular structure of the network was analysed, and the topological role of the different ponds was determined using the rnetcarto R package. To evaluate whether the structure of the network was significantly modular, null models with the oecosimu function of the vegan R package were performed. The algorithm used was quasiswap, with 2000 iterations. Finally, peripheral hubs and connectors were selected, those ponds that should be prioritized due to their greatest number of connections between modules (Guimera and Amaral, 2005).

3 Results

3.1 Invasion status

Bullfrog was recorded in 32 of the 75 sampled ponds in San Carlos, occupying a total area of 16.5 km^2. Seven ponds contained the highest bullfrog densities, eight had intermediate densities, and the remaining seventeen showed the lowest densities (Fig. 2). Invasion areas were located surrounding the

Table 1. Selected GLM models (with their explained deviance and adjustment quality) for richness and abundance of native post-metamorphic amphibians, in San Carlos. All the used exploratory variables are listed. For each selected exploratory variable, the estimated coefficient, the residual deviance, the P-value, and the effect (positive "+" or negative "–") are included. When a variable was not selected by the model it is indicated with a "NI" (not included). When a variable was not statistically significant it is indicated with a "NS". The sampling date was always included as an explanatory variable, because it denotes the experimental design.

GLM	Estimated coefficient	Residual deviance	9-value	Effect
Post-metamorphic richness (Explained deviance 13.4; Adjustment quality 1.02)				
(Intercept)	3.32			
Bullfrog presence	0.61	56.2	0.028	–
Air temperature				NI
Humidity				NI
Wind				NI
Cloud cover				NI
Sampling date		61.0	0.27	NS
Post-metamorphic abundance (Explained deviance 16.6; Adjustment quality 1.2)				
(Intercept)	0.20			
Bullfrog presence				NI
Air temperature	1.22	71.4	0.033	+
Humidity				NI
Wind	0.25	67.3	0.041	–
Cloud cover				NI
Sampling date		76.0	0.20	NS

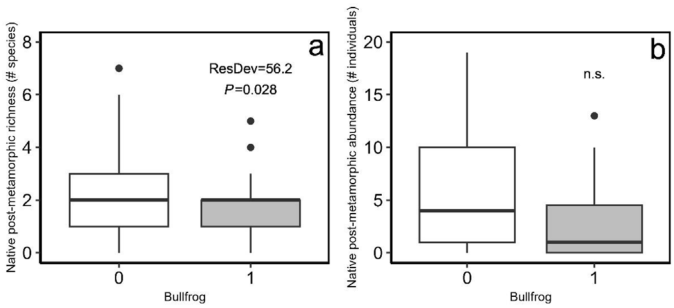

Fig. 3. Richness (A) and abundance (B) of native post-metamorphic anurans in relation to bullfrog presence(1)-absence(0) in San Carlos, Maldonado Department, Uruguay. White boxes indicate uninvaded ponds and grey boxes bullfrog invaded ponds. Inside the boxes the median is shown as a horizontal line. n.s: not statistically significant.

San Carlos stream and its tributaries, especially upstream of the La Paja creek. Also, the distribution of the bullfrogs was mainly observed near national and neighborhood roads, where there were more human settlements and more water reservoirs (Fig. 2).

3.2 Effects on native post-metamorphic amphibians

The native post-metamorphic amphibians recorded in the 60 water *bodies sampled were Boana pulchella, Pseudis minuta, Scinax granulatus, Scinax squalirrostris, Dendropsophus sanborni, Phyllomedusa iheringii, Leptodactylus luctator, Leptodactylus gracilis, Leptodactylus latinasus, Leptodactylus mystacinus, Odontophrynus asper Physalaemus gracilis, Pseudopaludicola falcipes, Rhinella arenarum and Rhinella dorbignyi*. The ponds' species richness was explained by a statistically significant GLM model (Tab. 1) that included bullfrog presence (residual deviance ResDev = 56.2; $P = 0.028$) and sampling date (ResDev = 61.0; $P = 0.27$) as explanatory variables. The native species richness was 1.9 ± 1.1 (mean ± standard deviation) in invaded ponds and 2.5 ± 1.9 in uninvaded ponds (Fig. 3a). A significant relationship between the abundance of post-metamorphic native anurans and the presence-absence of bullfrogs at the pond level was unable to be identified (ResDev = 75.9, $P = 0.87$) (Fig. 3b). The selected GLM model for post-metamorphic native anurans abundance (Tab. 1), included wind intensity (ResDev = 67.3, $P = 0.041$), air temperature (ResDev = 71.4, $P = 0.033$) and sampling date (ResDev = 76.0, $P = 0.20$).

The three most frequent anuran species showed greater abundance variation in uninvaded ponds than in invaded ones,

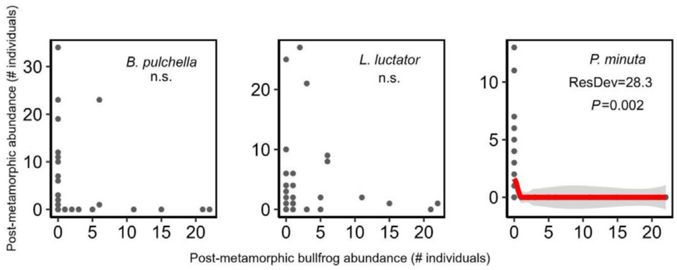

Fig. 4. Abundance of the three most frequently observed post-metamorphic native anurans (*Boana pulchella*, *Leptodactylus luctator*, and *Pseudis minuta*) in relation to bullfrog abundance in San Carlos, Maldonado Department, Uruguay. Black points are observations for each sampled pond, n.s. is not statistically significant, and the red line in the rightmost graph is the negative binomial GLM model (ResDev: Residual Deviance, P: P-value). The grey shade is the adjustment of the model for its standard deviation.

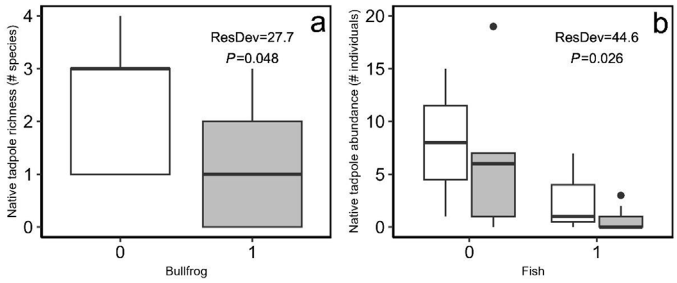

Fig. 5. Specific richness and abundance of native anuran tadpole assemblage in relation to bullfrog (A) and fish (B) presence(1)-absence (0) in San Carlos, Maldonado Department, Uruguay. White boxes refer to the uninvaded ponds and grey boxes to the invaded ponds. Inside the boxes the median is shown as a horizontal line.

and a tendency to decrease with increasing bullfrog abundances (Tab. 2). For instance, *P. minuta*'s abundance showed a statistically significant and abrupt decrease with an increase in bullfrog abundance (ResDev = 28.34; *P* = 0.002). However, this relation was not statistically significant for *B. pulchella* (ResDev = 40.00; *P* = 0.13) and *L. luctator* (ResDev = 52.06; *P* = 0.38).

3.3 Effects on native tadpoles

The native tadpoles observed in the 26 sampled ponds were *B. pulchella, P. minuta, Scinax* spp. (*S. granulatus* and/or *S. squalirostris*), *P. gracilis, Rhinella* spp. (*R. arenarum* and/or *R. dorbignyi*) and *Leptodactylus* spp. (*L. gracilis, L. latinasus* and/or *L. mystacinus*). The number of native tadpole species was significantly higher in uninvaded and more vegetated ponds. The best GLM model obtained, explained native tadpole richness in the studied ponds when including bullfrog presence and macrophyte cover as variables (Tab. 3). Both exploratory variables were statistically significant (bullfrog presence: ResDev = 27.7; P = 0.048 and macrophyte cover: ResDev = 21.2; P = 0.011). In all cases, larval richness increased with increased vegetation cover (coefficient = 1.01). Water bodies invaded by bullfrogs had lower tadpole richness than those that were not invaded (Fig. 5a).The average number of native tadpole species was 1.2 ± 1.1 (mean ± SD) in invaded ponds, and 2.3 ± 1.2 in uninvaded ones.

Native tadpole abundance was also affected by bullfrog invasion (Tab. 3). This abundance was explained by a model that included the following statistically significant explanatory variables: presence of bullfrog (ResDev = 44.6; *P* = 0.026), pond area (ResDev = 31.2; $P < 0.001$) and presence of fish (ResDev = 26.3; *P* = 0.026). Native tadpole abundance was significantly lower in systems with fish (4.7 ± 9.4) than in systems without fish (7.0 ± 7.4), and the same pattern was observed in bullfrog-invaded ponds (3.7 ± 6.0) than in uninvaded ones (10.9 ± 11.8) (Fig. 5b). Both in invaded and uninvaded ponds, pond area was also a determinant of abundance. For instance, the abundance of tadpoles was inversely related to the pond area (coefficient = 1.00), and it dropped steeply in ponds over 500 m^2.

3.4 Pond prioritization

The San Carlos pond network (mean area = 1026 m^2, range = 10 to 64262 m^2) was dense, highly interconnected and presented a strongly modular structure (modularity coefficient = 0.81; z = 387.1; *P*-value < 0.001). The invaded ponds belonged to four modules. The module that contained the area where the bullfrog farm was located, also contained 22 invaded ponds. The remaining six invaded ponds were located in three adjacent modules, at the edges of the mapped distribution (Fig. 6). A list of 55 ponds that should be prioritized for local management of the bullfrog invasion was obtained, due to its

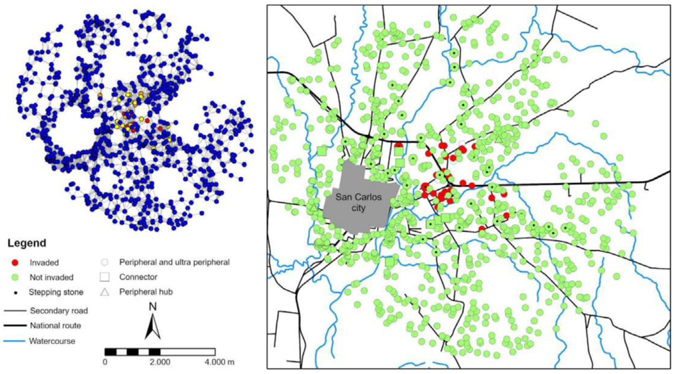

Fig. 6. Topological network of ponds of San Carlos, Maldonado Department (Uruguay), according to distances related to the dispersal capacity of *L. catesbeianus* (left). Uninvaded ponds appear in blue and those invaded by *L. catesbeianus*, in red, orange and yellow (according to their degree of invasion, described in methods). San Carlos' pond network map shows the stepping stones (black middle point), the connectors (square symbol) and the peripheral hubs (triangle symbols). Bullfrog invaded ponds are in red on the map (right).

role in the network (Electronic material, Tab. E1). Seven of those ponds that act as connectors and two as peripheral hubs were identified. Some connectors were located close to invaded ponds. Furthermore, all invaded ponds were identified as ultraperipheral or peripheral nodes. From the 5% of ponds with the highest betweenness values (*i.e.* stepping stones), five were already invaded by bullfrogs. A significant proportion of these stepping stones were located close to the invasion front (Fig. 6). The list of ponds ranked by high connectivity between modules or by their role as stepping stones, and further information about the pond network, were included as supplementary material (Electronic material, Tab. E1, Fig. E1).

4 Discussion

Our study presents the first report on the distribution, density, impacts on the native amphibian assemblage, and prioritization of management strategies for ponds invaded by bullfrogs in Uruguay. The observations and analyses determined that the bullfrog population is relatively spread out in San Carlos. This knowledge is relevant in the context of a strong need to clearly diagnose the status of the small invasive populations in southern South America, especially in order to address their management (Pluess *et al.*, 2012; Barbosa *et al.*, 2017; Schwindt and Bortolus, 2017). In this sense, our observations are an interesting contribution, which also confirms bullfrogś negative effects on local biodiversity (Li *et al.*, 2011). In addition, the pond network analysis revealed that modules and a series of nodes that would have an important role in the dispersion of this invasive species were identified.

The invasive bullfrog population studied herein is the largest population known in Uruguay. Its estimated distribution area was 1.9 km^2 (six ponds) in 2015 (Laufer *et al.*, 2018a), while the current area determined is approximately six fold larger, occupying nearly 16.5 km^2 (32 ponds). Before this study, the largest feral population in Uruguay was reported in Aceguá (Cerro Largo Department), where expansion began in 2012 (Laufer *et al.*, 2018a). Considering that bullfrog distribution in San Carlos is even greater than in Aceguá, we can assume that the San Carlos population is in an expansion phase.

Through unstructured surveys with local people, a second unregistered farm was noted to have been working in the area, maintaining and fattening animals from the main farm, Laguna Dorada (unpublished data). Therefore, there were potentially two bullfrog propagule sources approximately one kilometer apart. This additional farm could have influenced the current distribution. In fact, the high bullfrog density area is close to both old farms. Interestingly, dispersal could have occurred from both areas in the same direction, upstream over the highways and roads (Fig. 2). This association between bullfrog presence and human activities could have favored dispersal. Specifically, in the case of San Carlos, the existence of many artificial lentic systems and water reservoirs, may favor the establishment of this invasion and dispersion (Jeschke and Strayer, 2006). Considering that the bullfrog is not a very selective species, choosing all kinds of permanent ponds (Nie *et al.*, 1999), it is clear that its expansion is strongly affected by socio-economic activities. Ficetola and collaborators (2010) found that land use can explain the distribution of this species' invasion. Undoubtedly, the human dimension must be included in the research and management of invasive alien species to achieve significant progress in understanding this phenomenon (Peterson *et al.*, 2013; Ballari *et al.*, 2016).

Results show a negative impact of bullfrog invasion on post-metamorphic native amphibian richness. This effect coincides with the evidence reported from other regions (reviewed by Kraus, 2009; but see Both and Melo, 2015) and suggested in Uruguay (Laufer *et al.*, 2008; Lombardo *et al.*, 2016; Laufer and Gobel, 2017). The observed decline in species richness is expected at the site scale (pond) due to the invasion of a large and abundant top predator (Oda *et al.*, 2019). The impacts of a large predator can be drastic throughout the food web, and invasive predators often exert greater pressure than native predators (Sih *et al.*, 2010; Thomsen *et al.*, 2014). This difference may be due to the inability of native species to detect the new predator (Polo-Cavia *et al.*, 2010), or the use of other invasion weapons which are novel to the native community (Strauss *et al.*, 2012).

Table 2. GLM models (with their Explained deviance and Adjustment quality) for the abundance of the three most frequent native post-metamorphic amphibians, in San Carlos. For both exploratory variables, the estimated coefficient, the residual deviance, the P-value, and the effect (positive "+" or negative "–") are included. When a variable was not statistically significant it is indicated with a "NS". The sampling date was always included as an explanatory variable, because it denotes the experimental design. The sign "*" indicates a statistically significative effect of this factorial variable.

GLM	Estimated coefficient	Residual deviance	*P*-value	Effect
***Boana pulchella* post-metamorphic abundance (Explained deviance 50.4; Adjustment quality 0.73)**				
(Intercept)	0.015			
Observed bullfrog abundance		40.00	0.13	NS
Sampling date	*	42.26	<0.001	
***Leptodactylus luctator* post-metamorphic abundance (Explained deviance 22.9; Adjustment quality 0.95)**				
(Intercept)	5.7			
Observed bullfrog abundance		52.06	0.38	NS
Sampling date	*	52.86	0.002	
***Pseudis minuta* post-metamorphic abundance (Explained deviance 37.8; Adjustment quality 0.51)**				
(Intercept)	0.62			
Observed bullfrog abundance	$3.5\ E^{-9}$	28.34	0.002	–
Sampling date		37.74	0.051	NS

Although post-metamorph richness was statistically different in invaded and uninvaded systems, the effect of invasion on post-metamorph abundance was not significant. This lack of significance could be due to the great variation in abundance recorded in uninvaded systems. Furthermore, not all native anuran species are equally affected by bullfrogs. There may be species that are more tolerant and are, therefore, more abundant in invaded systems (Bucciarelli *et al.*, 2014). For this reason, the analysis of the most common species can contribute to the understanding of the system.

The most affected species was *Pseudis minuta*, which is characterized by its post-metamorphic aquatic habits. Although we observed a drop in the abundance of *Boana pulchella* and *Leptodactylus luctator*, it was not statistically significant. *Pseudis minuta* has very similar microhabitat use and morphological conformation to *L. catesbeianus*, but it is much smaller; *L. catesbeianus* mean adult male Snout-Vent Length (SVL) is 131 mm (Kaefer *et al.*, 2007), *P. minuta* mean adult male SVL is 31 mm (Melchiors *et al.*, 2004). This species would be much more exposed to predation than other species because of its aquatic habits, smaller body size, and probable inability to recognize a new exotic predator (Polo-Cavia *et al.*, 2010). Surely, the *Pseudis* genus of Neotropic aquatic frogs could be one of the genera most affected by bullfrog invasion in the region (Both and Melo, 2015; Silveira and Guimarães, 2021). This type of asymmetric effect, along with the encounter rate, have already been reported for the Northern Hemisphere (Pearl *et al.*, 2004) and Southeastern Brazil (Silva *et al.*, 2011). These asymmetries should be considered in the creation of management plans that seek to mitigate the impacts of this invasion.

The impacts of bullfrogs on native tadpoles were more noticeable than on post-metamorphs. This contrast is reasonable if the habits of the bullfrog as an invasive anuran are considered, and its greater interaction with aquatic organisms (Descamps and De Vocht, 2016). In addition to post-metamorph predation pressure, the existing evidence suggests that bullfrog tadpoles can also prey on eggs and small animals (Schiesari *et al.*, 2009; Ruibal and Laufer, 2012), and may even affect the development and survival of native tadpoles (Kiesecker *et al.*, 2001; Blaustein *et al.*, 2020; Tasker *et al.*, 2022).

Bullfrogs can coexist and also be benefited from predator fish (*i.e.* indirect trophic effects: Smith *et al.*, 1999; Semlitsch *et al.*, 2015), but they can also use other water habitats where fish are infrequent. The impacts of this invasion could be greater since bullfrogs show land dispersal and colonize water bodies where fish are absent or at low densities (Hecnar and M'Closkey, 1997). Thus, the ponds with restricted areas and/or hydroperiods, as well as suboptimal pH and oxygen conditions for fish can be invaded by bullfrogs (Descamps and De Vocht, 2016). All of these factors would severely restrict the availability of viable breeding sites for various native anurans, at the landscape level. Macrophyte coverage showed an important role in maintaining native tadpole richness. The importance of maintaining spatial heterogeneity, as a source of refuge and microhabitat for native species, and as a way to mitigate the impacts of the bullfrog invasion, should be emphasized. Macrophyte coverage favored the coexistence of a greater number of native species, as observed in the results (Hartel *et al.*, 2007).

Most empirical evidence about bullfrog effects comes from studies of post-metamorphic stages. Nevertheless, aquatic pond communities, including tadpoles and other organisms, seem to be strongly affected as well (Gobel *et al.*, 2019). Bullfrog invasions could affect the recruitment of species that depend on permanent ponds. They could even affect the ecosystem services associated with the aquatic systems in which they take place, at least in the long term (Moore and Hunt, 2012).

We must consider possible biases generated by the methodology used in the sampling, both of larvae and post-metamorphs, that could affect our results. The samplings were limited in time and seasons and therefore may reflect an underrepresentation (even an overrepresentation) of some amphibian species (*i.e.* Both *et al.*, 2009). To better understand the effects and explore the underlying mechanisms, it would be necessary to increase sampling effort.

Table 3. Selected GLM models (with their Explained deviance and Adjustment quality) for richness and abundance of native amphibian larvae, in San Carlos. All the used exploratory variables are listed. For each selected exploratory variable, the estimated coefficient, the residual deviance, the *P*-value, and the effect (positive "+" or negative "–") are included. When a variable was not selected by the model it is indicated with a "NI" (not included).

GLM	Estimated coefficient	Residual deviance	*P*-value	Effect
Tadpole richness (Explained deviance 34.6; Adjustment quality 0.98)				
(Intercept)	1.03			
Bullfrog presence	0.66	27.7	0.048	–
Pond area				NI
Fish presence				NI
Macrophyte cover	1.01	21.2	0.011	+
Tadpole abundance (Explained deviance 46.9; Adjustment quality 1.2)				
(Intercept)	72.63			
Bullfrog presence	0.12	44.6	0.026	–
Pond area	1.00	31.2	0.00026	–
Fish presence	0.25	26.3	0.026	–
Macrophyte cover				NI

The obtained pond web and prioritization are valuable to prevent bullfrog expansion. From the network analysis, a list of 55 ponds that were of great importance to prevent or delay the expansion process of *L. catesbeianus* in San Carlos was generated (Electronic material, Tab. E1). Most of the prioritized ponds are uninvaded yet, and then are sites where the arrival of bullfrogs should be avoided (*e.g.* physical, chemical, and other measures). The relatively small number of invaded ponds and their restricted allocations to four modules, indicate that eradication is still possible. Kraus (2009) reviewed previous bullfrog control actions, identifying five cases of successful eradication in Europe (Great Britain, Netherlands and Germany). The success of these actions was explained by the low number of invaded ponds (maximum 53) detected at early stages, and because different management techniques were applied (*i.e.* pond draining and/or fencing, removing adults and tadpoles by aquatic traps, pit-fall traps, hand-capture, shooting and electrofishing). In recent years, the eradication of a population in Yosemite National Park, United States of America, was also reported with the use of seine net for tadpoles, and hand catching and shooting for post-metamorphs (Kamoroff *et al.*, 2020). In addition, in Belgium, fyke nets have been used successfully to control bullfrogs (Louette *et al.*, 2013; Descamps and De Vocht, 2023). These efforts can be complemented with a series of promising new techniques that can amplify the results (*e.g.* Groffen *et al.*, 2019; Sutherland *et al.*, 2019; Everts *et al.*, 2022). Invaded pond distributions and position within the web, as well as bullfrog abundance at each pond, are useful data for planning this possible eradication in San Carlos. In fact, eradication should begin in the five ponds that have already been invaded by bullfrogs and were prioritized due to their high risk of regional dispersal (*i.e.* stepping stones).

Since the bullfrog is one of the priority species for control and/or eradication in Uruguay (Aber *et al.*, 2012), the available rapid response protocol should be applied to this population restricted to a relatively small area in San Carlos (Comité de Especies Exóticas Invasoras, 2018a). The information provided here is useful for the diagnosis and planification to the Specialized Technical Group for Rapid Response, of the National Committee for Invasive Alien Species of the Ministry of the Environment. This committee already has the experience of an action plan for another bullfrog focus in Aceguá, in Northeastern Uruguay (Comité de Especies Exóticas Invasoras, 2018b). Unfortunately, the actions implemented there were few, isolated, and lacked coordination and involvement of the local social actors (personal observations), underestimating not only the management methods but also the socio-environmental nature of the problem (Pluess *et al.*, 2012). This previous experience suggests that a successful bullfrog control in San Carlos would be difficult to achieve. In any case, we strongly recommend that the authorities implement eradication actions in San Carlos. This eradication must integrate multidisciplinary approaches, considering different interacting factors, such as scientific knowledge, social aspects, the ethics of wildlife management, as well as a fine knowledge of the invasion in the landscape context.

As a general conclusion, there is a significant risk of bullfrog expansion, with negative consequences to native San Carlos amphibians, that could be prevented. The information reported herein is essential to understand the situation and to predict scenarios and, therefore, achieve better conservation decisions. Our findings confirm the importance of maintaining strong monitoring and strengthening the determination of the actual distribution of invasive alien species (Simberloff *et al.*, 2005). Therefore, conservation efforts should aim to eradicate bullfrogs, and prevent its expansion based on field data analysis.

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Supplementary Material

Table E1: Ponds prioritized for bullfrog control, due to their high connectivity at the landscape level, in San Carlos, Maldonado (Uruguay). These are the priority ponds to attend to in an eradication plan; eliminating or preventing the arrival of the bullfrog would slow down the invasion process. For each pond, the geographic coordinates in UTM 21S, the invasion category of *L. catesbeianus* and the reason for its prioritization, are included.

Figure E1: Topological ponds network at San Carlos, Maldonado (Uruguay), according to distances related to the dispersal capacity of *L. catesbeianus*. The size of each node is proportional to the degree centrality value of each pond (top left). The size of each node is proportional to the value of betweenness centrality of each pond (top right). The squares represent the ponds with the topological role of connector, and the triangles represent the peripheral hubs (bottom left). In these first three, uninvaded ponds appear in blue and those invaded by *L. catesbeianus*, in red, orange and yellow (according to their degree of invasion, described in Methods). In the latter, the colors identify the different modules of the network. Invaded ponds appear in larger sizes (bottom right).

The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2023016/olm.

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