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Can Protected Areas with Agricultural Edges Avoid Invasions? The Case of Bullfrogs in the Southern Atlantic Rainforest in Brazil

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Bruno Madalozzo, Camila Both, and Sonia Cechin (2016) The American bullfrog, *Lithobates catesbeianus*, is one of the 100 most harmful invasive species of the planet. Climatic and topographic models predict that the Atlantic Forest regions of southern Brazil are favorable for the establishment of invasive bullfrog populations. The predicted increase of temperature and concentration of gases associated with the greenhouse effect will augment the vulnerability of protected areas of the Atlantic forest to bullfrog invasions in the coming years. In this study we investigated to what extent protected areas of the Atlantic Forest surrounded by anthropogenic landscapes are vulnerable to bullfrog invasions. We conducted surveys in 36 waterbodies located either in a protected area or in anthropogenically modified adjacent locations on a forest-edge-agriculture gradient. We collected data on abundance and breeding to identify the main descriptors (local and landscape variables) that explain the distribution of bullfrogs along this gradient. The variance partitioning analysis showed a strongest association of bullfrog abundance with local waterbody descriptors (area-depth-hydroperiod) and secondarily with a forest-edge-agriculture gradient, *i.e.*, the landscape. The observed distribution pattern suggests that protected areas are likely to be invaded by bullfrogs. Therefore, management strategies should focus on both scales: landscape and waterbody. Supervising the construction of large (permanent or deep) waterbodies in edge habitats of the park and adjacent areas can be effective and agriculture and forest management could importantly complement the prevention of invasions.

Key words: Invasive species, *Lithobates catesbeianus*, Conservation unit, Edge effect, Brazil.

BACKGROUND

Biological invasions and habitat loss are two major factors driving population declines and extinction of native species (Vitousek et al. 1997; IUCN 2015). These processes can affect native communities either separately or synergistically (Didham et al. 2007). The conversion of natural areas into anthropogenic landscapes causing habitat loss is a globally widespread and growing phenomenon, and has been recognized as the main cause for the loss of biodiversity (Vitousek et al. 1997). On the one hand, habitat loss could be the first step towards the establishment of invasive species, since a high disturbance frequency may weaken ecosystem functions and modify biological relationships facilitating the establishment of such species (Williamson 1996; Didham et al. 2007; Fuller et al. 2011). On the other hand, biological invasions may initiate habitat conversion, where the growth of a single invasive population can drive habitat loss (Drake et al. 1989; Vitousek et al. 1997). These two cases exemplify the difficulties of assessing the independent influence of factors that drive biodiversity loss (D’Antonio and Vitousek 1992; Vitousek et al. 1997).

Many studies have indicated a positive association between introduced species and anthropogenically disturbed areas, or areas that have lost their natural habitats (*e.g.*, Forman

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and Alexander 1998; Fuller et al. 2011). This positive relationship has often been attributed to the high disturbance frequency common to anthropogenic areas (Williamson 1996). Thus, the existence of buffer zones around protected areas would be desirable to attenuate disturbances for conservation purposes. According to Reid and Miller (1989), a buffer zone is defined as ''a collar of land managed to filter out inappropriate influences from surrounding activities''. However, some protected zones are surrounded by anthropogenically modified areas, and thus might be more susceptible to invasions (Vilà and Ibáñez 2011). Specific environmental conditions, such as increased light levels, higher temperature and wind exposure, along edges without buffer zones can facilitate the establishment of invasive species, allowing their dispersal into protected areas (Pickett and Cadenasso 1995; Harper et al. 2005).

During each of a series of stages of the invasion process, species must overcome challenges related to their own natural limitations to survive, reproduce and disperse (Blackburn et al. 2011). Each of these challenges can act as an environmental filter at different spatial scales. At large spatial scales, the climate can be the most important filter for species establishment in new locations (Hayes and Barry 2008; Bomford et al. 2009). Additionally, landscape and local habitat factors may constitute environmental challenges at smaller scales, even when the climate favors invasion success (Wang and Li 2009; González-Moreno et al. 2013; Garcia et al. 2015). For example, dense forest edges and closed canopies represent landscape attributes that can act as physical barriers against invasions or cause biologic disadvantages for invaders on a regional scale (Cadenasso and Pickett 2001; Knapp 2014). Locally, biotic interactions between invasive and native species can influence the invasion process (Blackburn et al. 2011).

The factors determining distributional and abundance patterns of most invasive species at different spatial scales are unknown. Hence, the development of management strategies to control invasive species and/or to promote the conservation of native species can be difficult. One such example is the invasive American Bullfrog *Lithobates catesbeianus*. This species is native to eastern portions of North America and has established populations in more than 40 countries (Ficetola et al. 2007). The bullfrog is widely distributed in its native area and has a large body size (Bury and Whelan 1984; Ficetola et al. 2007), traits that are expected to be positively associated with establishment success (Tingley et al. 2010). The species showed superior competitive capabilities when experimentally compared with other anurans (Kiesecker and Blaustein 1998; Kiesecker et al. 2001; Blaustein and Kiesecker 2002); and is a generalist predator, also consuming other anuran species (Hayes and Jennings 1986; Pearl et al. 2004). Moreover, potentially, bullfrog can be a vector of *Batrachochytrium dendrobatidis*, the chytrid fungus associated with global amphibian declines (Berger et al. 1998; Daszak et al. 2004). For these reasons, the bullfrog is considered to be one of the 100 most harmful invasive species on the planet (Lowe et al. 2000; GISD 2016). In Brazil, the species is widespread, but is more often found in southern and southeastern regions of the Atlantic Forest (Giovanelli et al. 2008; Both et al. 2011).

Predictive models based on land use and bioclimatic and topographic variables demonstrated that the southern regions of Brazil, mostly in the Atlantic rainforest, are favorable for the occurrence of *Lithobates catesbeianus* (Ficetola et al. 2007; Giovanelli et al. 2008). Both et al. (2011) reviewed the records of species occurrences for Brazil and expanded the number of municipalities with invasive populations from 80 (data from 2002 to 2008) to 130 (data from 2006 to 2011) (Giovanelli et al. 2008). According to Nori et al. (2011), predictive models based on climatic data and the potential distribution of bullfrogs indicated that for the near future (2050 and 2080), the conditions of the Brazilian Atlantic rainforest will remain favorable for the occurrence of bullfrog populations. In scenarios predicting higher temperatures and gas concentrations related to the greenhouse effect, protected areas of the Atlantic rainforest will become vulnerable to bullfrog invasions (Nori et al. 2011; Loyola et al. 2012).

Despite these modeling predictions, bullfrog populations are already present in several protected areas of the Atlantic rainforest in Brazil (*e.g.* Lucas and Fortes 2008; Dallacorte 2010; Both et al. 2011; Iop et al. 2011). There is a need for studies directed at the understanding of the spread and occupation processes of invasive bullfrogs on protected areas, which are considered crucial to the conservation of native species *in situ* (Chape et al. 2005). In this study, we investigated the effect of local and landscape factors on the distributional patterns of the invasive species *Lithobates catesbeianus*. The study was carried out within a protected area of the Atlantic Forest and its anthropogenically affected neighborhood, representing a forest-edge-agriculture gradient. Specifically, we sought to answer the question: what are the main local and landscape factors that explain the distribution of bullfrogs along a forest-edge-agriculture gradient? With this work, we aimed to understand the ecological factors that explain bullfrog occupation of protected areas and the effectiveness of forested protected areas as functional barriers against invasions in the Atlantic Forest.

MATERIALS AND METHODS

Study area

This study was conducted in Turvo State Park (between 27°17' and 27°10'S, 53°48' and 53°58'W, 100 - 400 m.a.s.l.) and adjacent areas belonging to the municipality of Derrubadas. The park is located in the extreme northwest of Rio Grande do Sul, Brazil and has an area of 17, 491 ha (Fig. 1a). The vegetation is characterized by semi-deciduous seasonal forest (Oliveira-Filho and Fontes 2000) and represents the largest remaining preserved area of this vegetation type in the state (SEMA 2005). According to Maluf (2000), the climate is subtropical sub-humid with dry summers. Temperatures are commonly above 22°C in the summer, and oscillate between -3°C and 18°C in the winter. The average annual rainfall is 1,665 mm with rainfall well distributed throughout the year (SEMA 2005).

The park is surrounded by two other protected areas in the Argentinean territory located west of the Uruguay River: the Provincial Moconá Park (about 1,000 ha) and the Yabotí International Biosphere Reserve (about 236, 613 ha). It is unlikely that the river acts as a significant biological barrier to aquatic organisms such as bullfrogs because regular flooding promotes dispersal (Achaval et al. 1979; Gudynas 1984). In contrast, within Brazilian territory, the park edge is well delimited and marked by intensive land use. Numerous rural properties are located next to the forest edges of the protected area, and there is no buffer zone to prevent the possible impacts caused by human activities (mostly cattle farms, and corn and soybean crops).

Sampling

Initially, we mapped two transects spanning approximately 10 km (Fig. 1b): both transects started in an agricultural area at a distance of 5 km from the park's edge, traversing more conserved areas, and extended 5 km into the interior of the Park's edge. For sampling, we selected waterbodies primarily based on their position on the gradient, *i.e.* covering the maximum possible diversity of distances from the edge, both inside and outside the park. We sampled 36 waterbodies, 17 inside the park and 19 in the surrounding areas. They included natural ponds (16, five outside and 11 inside the park), stream pools (four, all inside the park) and artificial ponds or dams (16, two inside and 14 outside the park).

All waterbodies were sampled twice, in November 2011 (austral Spring) and in March 2012 (austral Summer), looking for tadpoles in the daytime and for adults at night. Both bullfrogs and the native anuran species were recorded, as well as invertebrate aquatic predators and fish presence. Tadpoles and aquatic predators were sampled with dipnets (40 × 30 cm, 2 mm mesh) in distinct microhabitats (*e.g.*, near the edge of the waterbody within and outside of vegetation; in deep water near the center of the waterbody within and outside of vegetation) (Shaffer et al. 1994). A maximum of five dipnet sweeps was performed in each microhabitat. Since heterogeneity is positively correlated to waterbody depth and size, fewer microhabitats were sampled in small, shallow and non-vegetated waterbodies than in large, permanent and vegetated waterbodies. All samples were taken between 10 am and 7 pm. For surveying adults, we used visual and acoustic searches around the perimeters of breeding sites. Surveys began 30 min after sunset. The sampling effort for tadpole and adult surveys was proportional to size and habitat heterogeneity of the waterbody (Scott and Woodward 1994; Shaffer et al. 1994). Since heterogeneity is positively correlated to waterbody depth and size, less time was spent in small, shallow and non-vegetated waterbodies than in large, permanent and vegetated waterbodies. We stayed between 25 and 35 min at each site to estimate adult abundance, even when no individual was visually located or heard calling. Voucher specimens were collected and deposited at the Coleção Científica de Herpetologia da Universidade Federal de Santa Maria (ICMBIO permit N° 28322-1). Fish presence/absence was recorded based on visual inspection and/or interview with the rural owners and the park staff.

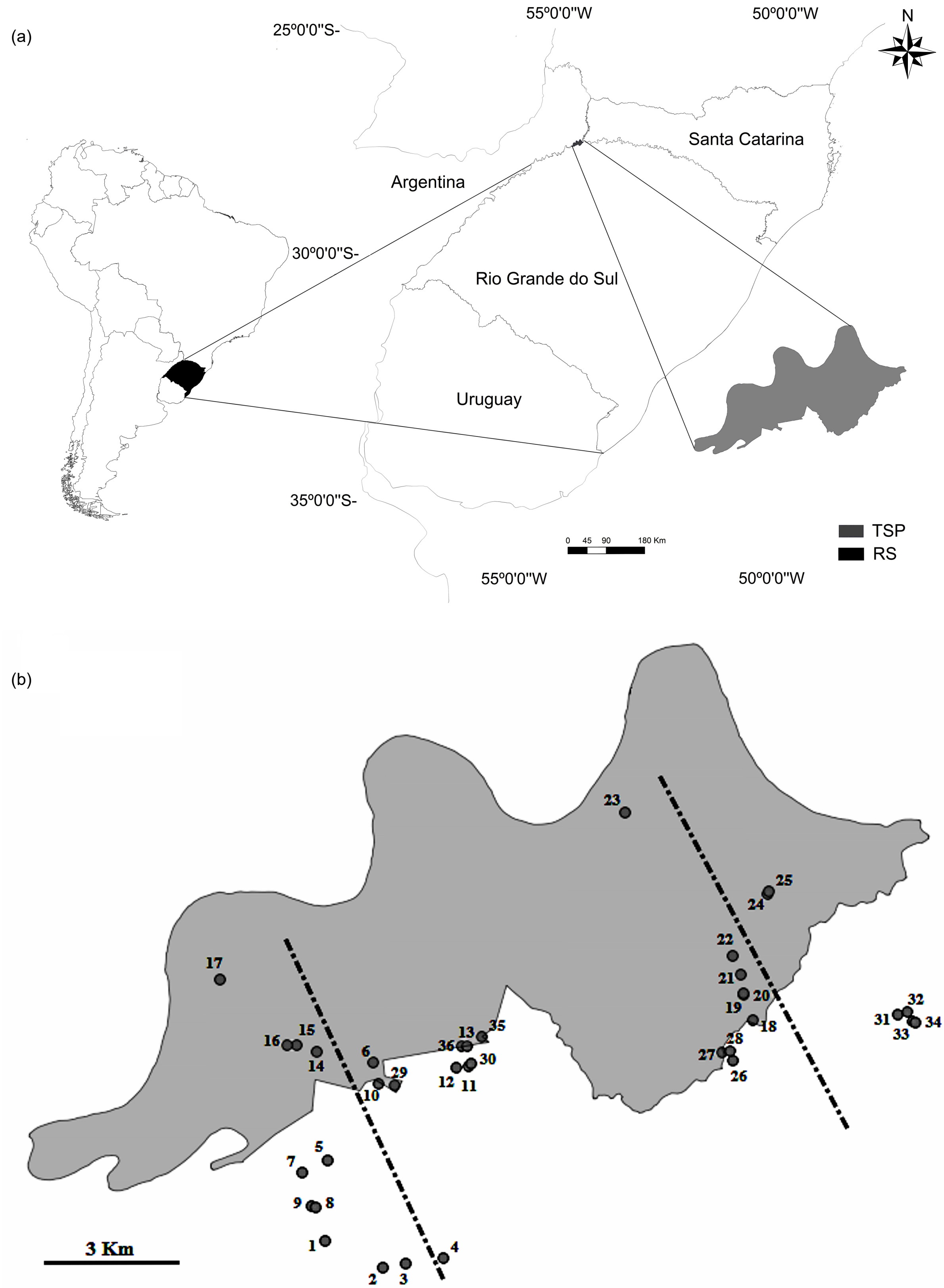

Fig. 1. (a) Location of Turvo State Park (TSP) in the far northwest of Rio Grande do Sul state (RS), Brazil. (b) Sampling design in Turvo State Park and surroundings areas: circles and respective numerations represent sampled waterbodies; dashed lines delineate the two transects that represent the spatial-environmental gradient of sampled breeding sites.

Spatial models and environmental descriptors

As the invasion process depends on environmental filters of invaded areas in distinct scales, we separated the environmental descriptors of bullfrog sampled habitats in three set of predictors: local factors, landscape factors and purely spatial factors. The predictors considered as landscape descriptors are represented by large scale measurements of each waterbody related to landscape elements of study area, as follow: distances of each waterbody from the forested edge of the park (square root transformed). The park edge is the zero point and distances inside the park were noted as negative distances, while distances outside the park were recorded as positive distances. We also considered distance to roads, and additionally whether the waterbodies were located within (forest matrix) or outside the park (agricultural matrix). We opted to use both distance from the edge and habitat type as descriptors because we had no previous ideas if we would have a continue variation of bullfrog abundance regarding the distances, or if only the habitat difference (inside-outside the park) would account for variation in abundances. The distances were calculated using Quantum GIS (Quantum Gis Development Team 2009). Local descriptors were represented by measurements in small scale related to abiotic and biotic factors of waterbodies, as follow: water surface area and depth (m); hydroperiod (temporary or permanent); percentage of vegetation cover (< 30%, 30-60%, > 60%, visually estimated); number of structural hydrophyte types (submerged, emergent and floating); number of structural vegetation types on the banks (grasses, shrubs, trees); invertebrate predators relative density (total abundance divided by number of sampled microhabitats) and fish presence (see supplementary data). Area measurements were made with a metric tape, and a GPS when necessary. Mean, minimum and maximum water depth was based on measurements made in each microhabitat sampled for tadpoles.

A third set of predictors was the purely spatial descriptors. They are orthogonal vectors describing the spatial arrangement of all waterbodies, which could affect bullfrog abundances through their respective proximities or distances (*i.e.* spatial autocorrelation). We utilized Moran's Eigenvector Maps (MEMs) to describe the spatial structure at multiple scales (Dray et al. 2006). The MEMs provide independent linear variables that represent spatial structures at all scales that could be perceived in our sample design (Borcard et al. 2011). For the construction of the MEM model, we utilized geographic coordinates for each site, collected with a GPS (Garmin eTrex H). The analysis resulted in six orthogonal spatial filters, which can be utilized as spatial predictors in further analysis. The truncation distance (minimum distance that connects all sites) among sampling sites was 7.2 km. The spatial filters were obtained using SAM software (Spatial Analysis in Macroecology), freely available from www.ecoevol.ufg.br/sam (Rangel et al. 2006). We used a spatial correlogram, which describe the magnitude and direction of spatial autocorrelation through Moran's I coefficient (Rangel et al. 2006), to inspect the relationship between spatial predictors and bullfrog abundance data (see Results).

Statistical analysis

Total bullfrog abundance was indexed as the highest number of individuals observed in a single sampling event. We considered both visual and acoustic records, taking care to not duplicate records of visually detected individuals that were also recorded as calling. We utilized log (x + 1) transformed data of total adult bullfrog abundance in the following analyses due to large deviations from normality. Also, before the analyses, we inspected for multicollinearity between predictors using Spearman correlations and excluded the predictors that was highly correlated (Spearman´s correlation above 0.70 or below -0.70) (see Table 1).

We used a variance analysis (ANOVA-one-way) to compare bullfrog abundances inside and outside the protected area, and a Chi-square test to evaluate if bullfrog frequency of occurrence differences among waterbodies in both areas. To investigate how bullfrog abundance is related to our predictors we used Generalized linear models (GLM; Nelder and Wedderburn 1972; Mccullagh and Nelder 1983) followed by a variance partitioning analysis. First, we investigated the relationship between bullfrog abundances and each group of predictors separately (local, landscape and spatial descriptors), using GLMs. The models were built by a stepwise forward procedure, where significant predictors are progressively selected (Zar 1999). This analysis selected the variables, which were used in the final GLM model for each group of predictors. Further, using the selected predictors from each set, we built a full model to understand the relationship between bullfrog abundance and the three groups of predictors combined (GLM, forward stepwise), followed by a variance partitioning analysis (Borcard et al. 1992). The partition of variance was employed in order to assess how space, landscape and local factors interact to determine bullfrog abundance, evaluating the independent and shared variance explained by the distinct groups of predictors (Legendre and Legendre 1998). The variance partitioning method is based on decomposing the total variance (R^2) to obtain unique fractions explained by each dependent variable or group of predictors. In this study, these components include local, landscape and space descriptors. All analyses were performed in the R environment (R-Development Core Team 2015).

RESULTS

The presence of *Lithobates catesbeianus* was observed in 26 of the 36 sampled waterbodies. Of these, nine (of a total of 17) were located inside the park and 17 (of a total of 19) in the adjacent agricultural areas (see the supplementary data). Where bullfrogs were present, their indexed relative abundance ranged from 1 to 123 (inside the park: X = 11 ± 29.8; outside: 11.7 ± 11.3 postmetamorphic individuals only), and bullfrog abundance was distinctly higher outside the park ($F = 7.01$, $p < 0.05$). We registered established populations (*i.e.*, presence of tadpoles, calling males or eggs) at 17 of the 36 waterbodies. The frequency of established bullfrog populations was lower in the forested area (0.23; 4 out of 17 waterbodies) than in the adjacent agricultural area (0.68; 13 out of 19 waterbodies), but they did not differ from the expected by chance (Chi-square = 2.7095, $p = 0.15$).

Only one spatial filter describing the distance between waterbodies was selected in the spatial model ($R^2 = 0.16$; $F_{2.34} = 7.77$; $p < 0.05$; Table 2). This filter represents the positive autocorrelation of bullfrog abundance for waterbodies within a distance of about 3.5 km, and negative autocorrelation for waterbodies within a distance of 7 km or more (Fig. 2). Therefore, in waterbodies within distances of up to 3.5 km, bullfrog abundance tended to be similar, while abundance differed among waterbodies at distances of 7 km or more.

The distance to the forest edge explained better the abundance variation than habitat type and road distance and it was the only predictor selected in the landscape model ($R^2 = 0.19$; $F_{2.34} = 8.43$; $p < 0.05$; Table 2). Abundance in waterbodies located towards the interior forest tended to be lower than abundance in locations near the edges or towards the agricultural areas (Fig. 3). Furthermore, bullfrog abundance was explained by two of 10 local variables: water surface area and hydroperiod ($R^2 = 0.59$; $F_{3.33} = 23.9$; $p < 0.001$; Table 2) (Figs. 4a and b, respectively). Abundances were higher in permanent waterbodies with larger areas.

Table 1. Spearman correlations coefficients between all local descriptors of waterbodies sampled inside and outside of protected area. Coefficients highly correlated (above 0.70 or below -0.70) were underlined

			Correlation coefficients											
	Mean	St. error	PVC	HP	WSA	NVB	NHT	WT	MD	MIND	AD	AIP	NSR	FP
HP	0.7778	0.42164	-0.218266	1.000000	0.147124	-0.013710	0.000000	0.033408	0.550526*	<u>0.800187*</u>	0.490622*	-0.196805	0.465283*	0.401859*
WSA	2.6404	0.68611	0.108800	0.147124	1.000000	0.041047	0.231799	-0.392695*	0.344454*	0.109890	0.204050	-0.251220	0.547610*	0.116674
NVB	1.6111	0.54917	0.096306	-0.013710	0.041047	1.000000	0.400091*	-0.051299	-0.076637	-0.153161	-0.015784	-0.022484	0.098179	-0.100868
NHT	1.5000	0.91026	0.696598*	0.000000	0.231799	0.400091*	1.000000	-0.603510*	-0.199082	-0.157006	-0.137473	0.111085	0.301873	-0.161086
WT	101.6667	0.67612	<u>-0.730265*</u>	0.033408	-0.392695*	-0.051299	-0.603510*	1.000000	0.054435	-0.002876	0.061450	-0.231216	-0.472703*	0.115663
MD	71.9194	37.83177	-0.282698	0.550526*	0.344454*	-0.076637	-0.199082	0.054435	1.000000	0.697970*	<u>0.854402*</u>	-0.217530	0.255473	0.316806
MIND	7.9528	5.38779	-0.276938	<u>0.800187*</u>	0.109890	-0.153161	-0.157006	-0.002876	0.697970*	1.000000	0.686167*	-0.141236	0.324893	0.225493
AD	36.6644	16.31958	-0.324090	0.490622*	0.204050	-0.015784	-0.137473	0.061450	<u>0.854402*</u>	0.686167*	1.000000	-0.187229	0.119273	0.346812*
AIP	5.2861	6.63145	0.379869*	-0.196805	-0.251220	-0.022484	0.111085	-0.231216	-0.217530	-0.141236	-0.187229	1.000000	0.010749	-0.301156
NSR	3.4722	2.44349	0.139986	0.465283*	0.547610*	0.098179	0.301873	-0.472703*	0.255473	0.324893	0.119273	0.010749	1.000000	0.236699
FP	0.3611	0.48714	-0.447016	0.401859*	0.116674	-0.100868	-0.161086	0.115663	0.316806	0.225493	0.346812*	-0.301156	0.236699	1.000000
PVC	45.1389	41.56726	1.000000	-0.218266	0.108800	0.096306	0.696598	-0.730265*	-0.282698	-0.276938	-0.324090	0.379869	0.139986	-0.447016*

Single asterisk (*) indicate statistically significant values of $p \leq 0.05$. PVC: percentage vegetation cover; HP: hydroperiod; WSA: water surface area; NVB: number of vegetation types in banks; NHT: number of structural hydrophyte types; WT: waterbodies type; MD: maximum depth; MIND: minimum depth; AD: average depth; AIP: invertebrate predators relative density; NSR: native species richness; FP: fish presence.

The full model combining spatial, landscape and local predictors accounted for 65% of the variation in bullfrog abundance (R^2 = 0.65; $F_{4.36}$ = 3; p < 0.001). Variance partitioning analysis revealed that 10.6% of the total variance in species abundance resulted from synergistic effects between the three predictor groups considered (Fig. 5). Bullfrog abundance was mainly determined by local factors (43%). The spatial and landscape descriptors only explained a small independent amount from the variance (1.1% and 1.6%, respectively). However, the spatial arrangement of the landscape, interior forest-edge-outer forest, accounted for 15.1% of the variation. This result indicates that the selected spatial filter is actually a filter describing the forest-edge- outer forest gradient. In addition, the synergistic effects of space and local factors explained 13.2%.

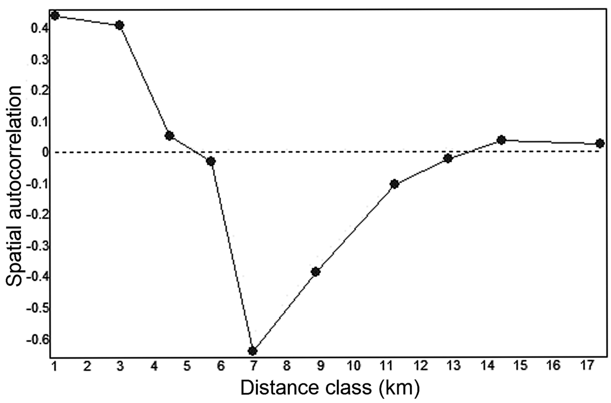

Fig. 2. Spatial filter representing the spatial autocorrelation (Moran's I) related to the distributional pattern of *Lithobates catesbeianus* measured in multiple classes of distance (km) between sampled waterbodies.

DISCUSSION

For a variety of taxa, forest edges or other edges between distinct vegetation types can function as physical and/or biotic barriers that inhibit the flux of invaders to the interior (Cadenasso and Pickett 2001; Holway 2005). However, the edges of forested areas can be even more susceptible to invasions when surrounded by highly disturbed areas (Debinski and Holt 2000). When comparing waterbodies inside and outside of the park, bullfrogs were found more frequently in the agricultural areas outside. Despite this, we observed higher bullfrog abundance in forest

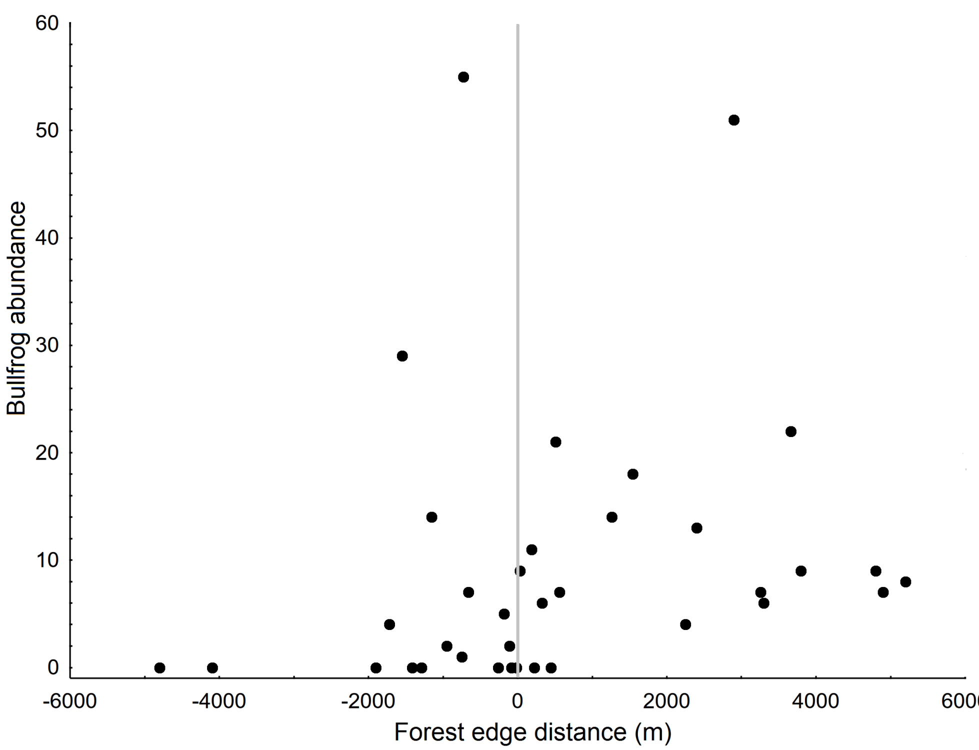

Fig. 3. Relationship between bullfrog abundance and forest edge distance (m) in a forest-edge-agriculture gradient. Negative values represent the distances between waterbodies within protected areas and positive values represent adjacent agricultural areas. Forest edge is represented by light gray line (or number zero).

areas adjacent to the forest edge. Our results indicate that the protected area is likely to be a weak barrier to bullfrog dispersal because they can occur at high abundances near the forest edge and still be present at areas inside the forest, although at lower abundances. Nevertheless, permanent waterbodies with invasive populations located close to the edge can facilitate invasions across the forest matrix, allowing bullfrog dispersal and breeding to the protected area (see Youngquist and Boone 2014).

Edge distance was related to bullfrog abundance, which was lower within the park and higher in edges and surrounding areas. In our study, edge distance and spatial structure were intrinsically correlated and, as expected, together explained a larger part of the variance in the distribution of bullfrog abundance on the edge gradient. Thus, waterbodies close to each other along the forest gradient (inside and outside of the park) influenced the pattern of bullfrog invasion towards the protected area. The edge effect on the distribution

Table 2. GLM model results relating bullfrog abundance with I) local, II) landscape and III) spatial descriptors, and IV) all selected predictors (from models I – III) combined. Significant selected descriptors from models I to III are distinguished by *

I) Local descriptors				
	Adj. R^2	*d.f.*	*F*	*P*
***Hydroperiod		1	13.67	< 0.001
***Water surface area (square root)		1	27.42	< 0.001
Number of vegetation types in banks		2	1.39	0.26
Number of structural hydrophyte types		3	1.17	0.26
Mean depth		1	3.53	0.06
Native species richness		1	0.29	0.59
Invertebrate predators relative density		1	1.78	0.19
Fish presence		1	1.33	0.25
Waterbody type		2	0.33	0.71
***Whole model	0.56		23.91	< 0.001
II) Landscape descriptors				
	Adj. R^2	*d.f.*	*F*	*P*
**Edge distance (square root)		1	8.43	0.0064
Road distance (square root)		1	2.23	0.14
Habitat type		1	0.10	0.75
**Whole model	0.19		8.43	0.0064
III) Spatial descriptors				
	Adj. R^2	*d.f.*	*F*	*P*
Spatial filter n° 1		1	2.65	0.11
**Spatial filter n° 2		1	7.77	0.0085
Spatial filter n° 3		1	1.17	0.28
Spatial filter n° 4		1	0.28	0.59
Spatial filter n° 5		1	3.44	0.07
Spatial filter n° 6		1	0.10	0.75
**Whole model	0.16		7.77	0.0085
IV) Full model with main descriptors selected from each set				
	Adj. R^2	*d.f.*	*F*	*P*
***Water surface area		1	29.2	< 0.001
***Hydroperiod		1	8.27	< 0.001
*Edge distance		1	5.37	< 0.05
Spatial filter n° 2		1	1.02	0.31
***Whole model	0.65		19.8	< 0.001

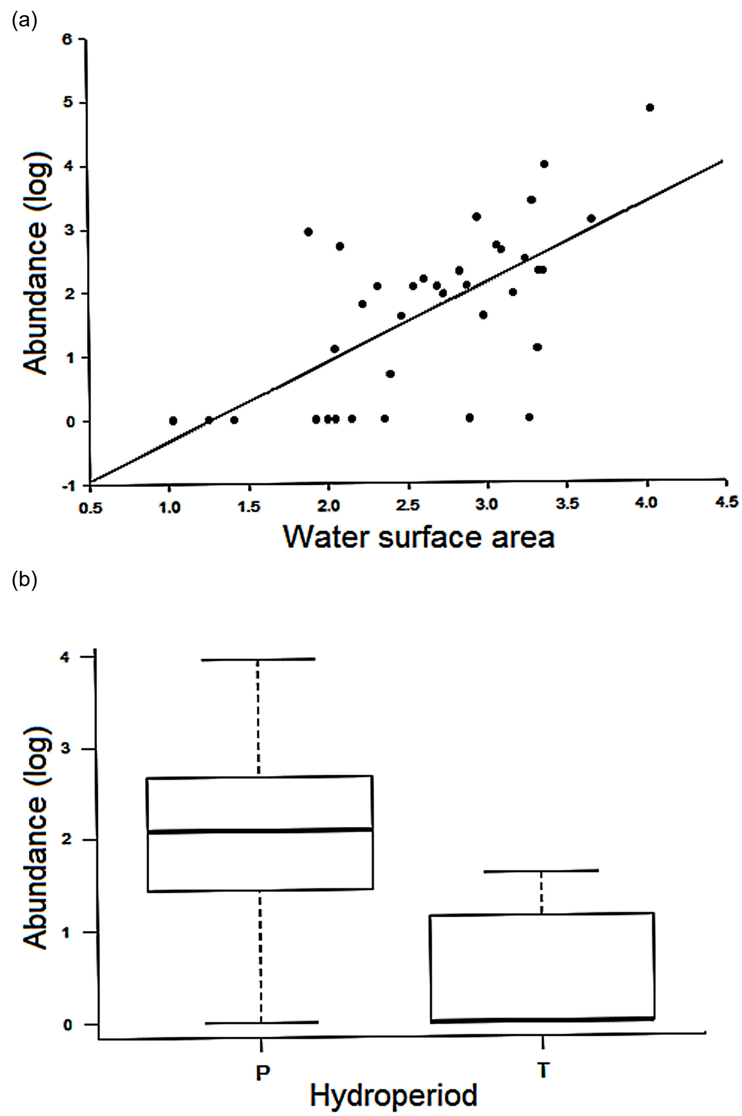

Fig. 4. Relationship between *Lithobates catesbeianus* abundance (transformed in logarithm) and local descriptors selected by the model: (a) water surface area (m^2) and (b) hydroperiod (P - permanent; T - temporary).

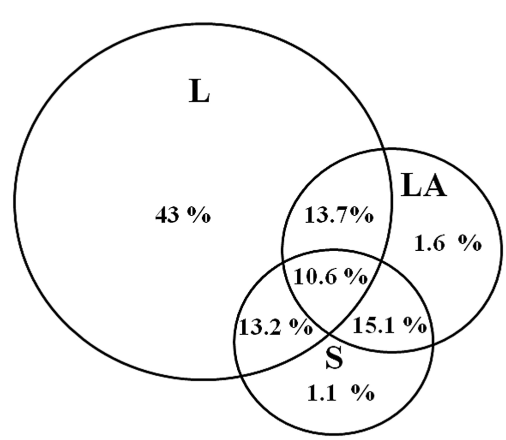

Fig. 5. Variance partitioning analysis. Venn diagram showing the independent and shared variance explained by local factors (L), landscape descriptors (LA) and spatial structure (S) related to *Lithobates catesbeianus* abundance.

of bullfrog abundance could be explained by the landscape configuration and permeability. Land use in adjacent areas have already been recognized as relevant factors for the distribution of many invasive species (Hansen and Clevenger 2005; González-Moreno et al. 2013). For bullfrogs, it has been recognized that they can benefit from open and/or disturbed habitats (Bury and Whelan 1984; Both et al. 2014). Additionally, bullfrogs appear to be less hindered by abiotic changes in edges between forest and agriculture areas (Youngquist and Boone 2014) and our results pointed out that bullfrogs are able to live within protect forest sites.

Due to the edge effect, invasive species richness and abundance tend to decrease with distance from the forest edge inserted in agricultural matrices (Dawson et al. 2015). Edges tend to be suitable for generalist species due to their specific environmental conditions, including light, humidity, temperature and wind (Harper et al. 2005). Such conditions, and the associated availability of resources, are progressively less common inside the protected areas. For this reason, one could argue that bullfrog dispersal to the park could have stabilized. In fact, bullfrog occurrence in Turvo Park was first noticed approximately 15 years ago, and most current records are in the same waterbodies as those reported a decade ago (SEMA 2005). Nevertheless, bullfrogs might still be spreading inside the park across habitat portions with low structural complexity, such as roads and streams similar to other invasive anuran species (see Brown et al. 2006). For instance, we recorded bullfrog juveniles in streams adjacent to the edges of the park. Although uncommon, the presence of bullfrogs in this type of waterbody has been recorded at higher latitudes in Brazil, and in some disturbed rivers in the United States (*e.g.*, Afonso et al. 2010; Fuller et al. 2011).

In this study, we found that the current distribution of bullfrogs in Turvo State Park and its surrounding areas was determined primarily by local variables, independently of spatial and landscape factors. The selected local variables (water surface area and hydroperiod) were positively correlated with bullfrog abundance. Our results corroborated those of Both (2012), who showed that local waterbody descriptors are the main determinants of bullfrog presence and abundance in Atlantic Forest sites, and that abundance tends to be higher in the deepest waterbodies. Waterbody features like area, depth and hydroperiod tend to be strongly correlated (Leibowitz and Brooks 2008). More permanent waterbodies are generally large and deep (or just deep in some cases), and are suitable to support bullfrog populations (Sepulveda et al. 2015; present study). The impact of local waterbody descriptors on bullfrog distribution can be attributed to bullfrog life history traits. The species spends much of its life in these habitat types, at larval and adult stages (Bury and Whelan 1984). Bullfrog tadpoles seem to lack plasticity to cope with variable hydroperiods, and thus depend on the permanence of waterbodies to reach metamorphosis (Cook et al. 2013). Additionally, permanent waterbodies with large areas may provide more options of refuge to bullfrogs when disturbed by some predator (Smith 1961). The availability of these large and permanent waterbodies inside the park is related to the establishment of populations into the protected area.

According Liu and Li (2009), the establishment of bullfrog populations in wild waterbodies is correlated with water permanency and presence of simple enclosures in bullfrog farms (or the absence of none, as our case). We have no information about when and how bullfrogs were introduced in the protected area, but probably the invasion process starts in permanent waterbodies in adjacent agricultural areas lacking any barrier. Distinctly, small waterbodies with high water level fluctuation decrease bullfrog resources and potentially cause clutch desiccation, and therefore they can hamper bullfrog establishment and function as abiotic filters against invasion (Bury and Whelan 1984; Wang and Li 2009). Accordingly, the suppression of permanent waterbodies in agricultural areas close to the protected area can be important measure to manage bullfrog populations inside the park (Wang and Li 2009; Youngquist and Boone 2014).

Biotic interactions also have the potential to act as filters on a local scale and to affect *L. catesbeianus* populations (but see Both et al. 2014). The biotic resistance theory proposed by Elton (1958) predicts that interspecific interactions in more diverse communities are strong and stable, which in turn would prevent or hamper invasions. In this study, native species richness was higher in permanent and large waterbodies (see Table 1), where the bullfrogs tend to show higher abundances. That is to say that species richness was positively related with bullfrog abundance, and therefore, it is unlikely that anuran diversity is working as a filter (Both et al. 2014). This invasive species has a high fecundity and fast sexual maturation (Kaefer et al. 2007). Perhaps for this reason the species can easily overcome many of the barriers related to biological interactions. For instance, one could expect that fish presence would negatively affect bullfrogs. And in fact, fish presence was also more frequently observed in permanent waterbodies, where bullfrogs reach their highest abundances (see Supplementary data). In addition, it is known that bullfrogs are generalist predators (Wang and Li 2009). Some studies also pointed out its superior predatory capabilities in comparison to native anurans, during both larval and adult life cycle stages (Kiesecker and Blaustein 1998; Kiesecker et al. 2001). Therefore, our results corroborate the findings of Both et al. (2014), which showed that local factors related with waterbody features are the most important filters to bullfrog populations in Atlantic Forest sites, but our study indicates that this will continue to be valid even inside preserved areas.

CONCLUSIONS

Our data demonstrated that bullfrogs respond to an environmental forest-edge-agriculture gradient. However, this gradient is less important than the area-hydroperiod-depth gradient that typically affects the structure of populations and/or communities inhabiting lentic waterbodies. Therefore, management strategies for populations of aquatic invaders, such as bullfrogs, should equally focus on landscape management, including buffer zones along forest edges, and on the management of waterbodies in the edge zone. An efficient way to hinder bullfrog invasions could be to avoid the construction of large-sized waterbodies (or deep and permanent waterbodies) in edge habitats and in surrounding protected areas.

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