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Landscape use and Habitat Configuration Effects on Amphibian Diversity in Southern Brazil Wetlands

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Abstract

In this study, we evaluated the effect of changes in natural wetlands on the amphibian diversity at differing spatial and temporal scales. We sampled 10 wetland sites along floodplains in southern Brazil. We classified the sites as reference or altered ponds according to the preservation degree and presence of human impact. The amphibian monitoring was conducted through calling surveys performed between 2015/2016 using an automated recording system that identified the calling male species. We identified 23 species, mainly distributed in the families Hylidae (43%) and Leptodactylidae (34.8%). The altered ponds had lower diversity and higher species dominance. Even ponds with the greatest landscape change revealed a high degree of resilience concerning the amphibian species composition. However, only *Boana pulchella* was dominant in altered ponds and *B. pulchella* and *Pseudopaludicola falcipes* were dominant in reference ponds. A reduction of amphibian richness was driven by the expansion of the urban area and loss of flooding areas. From 1999 to 2016 all sampled sites had their wetland area reduced as the surrounding urban area increased, contributing to the combined loss of habitat and reproductive sites of anurans in subtropical wetlands.

Keywords Anurans · Floodplain · Habitat loss · Riparian forest · Wetland

Resumo

Neste estudo, avaliamos o efeito das mudanças nas áreas úmidas naturais sobre a diversidade de anfíbios em escalas espaciais e temporais. Amostramos 10 áreas úmidas ao longo de várzeas no sul do Brasil. Classificamos as áreas como lagoas de referência ou alteradas de acordo com o grau de preservação e presença de impactos antrópicos. O monitoramento dos anfíbios foi realizado por meio de levantamentos de vocalizações entre 2015/2016, utilizando um sistema de registro automatizado que identificou as espécies de anfíbios machos vocalizadores. Identificamos 23 espécies, distribuídas principalmente nas famílias Hylidae (43%) e Leptodactylidae (34,8%). As lagoas alteradas tiveram menor diversidade e maior dominância de espécies. Mesmo as lagoas com maior mudança na paisagem revelaram uma resiliência expressiva em relação à composição

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de espécies de anfíbios. No entanto, apenas *Boana pulchella* foi dominante em lagoas alteradas e *B. pulchella* e *Pseudopaludicola falcipes* foram dominantes em lagoas de referência. As mudanças na paisagem favoreceram a redução da riqueza de anfíbios impulsionada pela expansão da área urbana e perda de áreas alagáveis. De 1999 a 2016 todos os locais amostrados tiveram suas áreas úmidas reduzidas à medida que a área urbana do entorno aumentou, o que é um cenário desfavorável para a manutenção da biodiversidade. Este diagnóstico revela a perda combinada de habitat e locais reprodutivos de anuros em áreas úmidas subtropicais.

Introduction

The conversion of natural areas is one of the human actions that generate the greatest impacts on natural ecosystems (Fuller et al. 2005; Becker et al. 2007, 2010; Hayes et al. 2010). Landscape change to meet the constant demands for the expansion of agricultural areas, as well as urban and industrial centers, generates a simplification and reduction of the landscape's heterogeneity. This process is usually followed by biodiversity loss and has been observed worldwide (Fuller et al. 2005; Fracetto et al. 2013). One of the most emblematic examples of this process is the suppression of tropical forests (Craig et al. 2008). However, many other ecosystems, such as savannas, grasslands, subtropical forests and wetlands, have been suffering intense human pressure in the last centuries and, as a result, have been losing a significant portion of their biodiversity. It is worth highlighting that wetlands are among the ecosystems suffering the highest impact (Revenga et al. 2005), with a rate of area loss superior to that of many tropical forests, for example (RAMSAR 2018). This situation is alarming since wetlands are among the most productive ecosystems and with the highest biological diversity on the planet (RAMSAR 2018).

The suppression of wetlands is ruled predominantly by local interests in increasing agricultural and commercial activities (Gopal et al. 2000; Cayuela et al. 2015). However, one of its consequences is the compromise not only of regional biodiversity but also of ecosystem services, especially those related to water supply (Millennium Ecosystem Assessment 2005), which are of great global relevance. This scenario makes wetlands priority ecosystems for study and conservation (Harris et al. 2005; Jantke and Schneider 2010) which also applies to similar ecosystems as floodplains (RAMSAR 2018). Since wetland loss is a worldwide phenomenon, some actions for the restoration of these habitats, such as the construction of artificial ponds, have been implemented, especially in the northern hemisphere (Brown et al. 2012). However, the lack of natural reference wetlands is a limitation for better planning of this process of wetland reconstruction (Drayer and Ritcher 2016). This highlights the importance of monitoring the remaining wetlands, especially those subjected to some type of threat (Hartel and von Wehrden 2013). In Brazil, large areas of wetlands have already been replaced or share space with highly degraded areas. Many of them are surrounded by urban areas with a continuous and disordered expansion without the implementation of a sewage treatment system. Brazilian wetlands originally occupied an area of about 5.3 million hectares and only about 3 million hectares remain today (Maltchik 2003; Carvalho and Ozorio 2007). Despite this unsettling reality, about 20% of the Brazilian territory still present wetlands (Junk et al. 2011), which are concentrated mainly in the Center-West and South regions of the country (Ministério do Meio Ambiente 2013).

The wetlands in the Brazilian South are the less studied ones regarding their fauna despite their territorial extension, comprising one of the largest wetland areas in South America. These wetlands consist of small ponds, i.e., water bodies smaller than 8 ha (Maltchik 2003). This pattern is the result of a long process of habitat fragmentation caused by agricultural expansion, especially of irrigated rice (Gomes and Magalhães 2004). Economic exploitation generates changes in the hydrological cycle of wetlands, which in general leads to a decrease in the time of water permanence (shortening of the flood season) (Cayuela et al. 2015), affecting several species, among which we can mention plants, birds and amphibians. Regarding amphibians, even species that use temporary ponds for reproduction and could establish in areas where the water bodies are ephemeral suffer from the impact of such changes (Deoniziak et al. 2017). Wetland-associated amphibians are considered good models to study the effect of changes in land cover on habitats (Curado et al. 2011), as their biphasic life history makes them sensitive to changes in pond and water availability in the habitat (Stoate et al. 2009; Becker et al. 2010; Tryjanowski et al. 2011). They are also sensitive of changes in the structure of the aquatic vegetation (Burkett and Thompson 1994; Watson et al. 1995) and many other habitat modifications (Gibbs et al. 2005; Hartel et al. 2009; Simon et al. 2009). Finally, urbanization is often associated with the establishment of roads or highways, which decrease amphibian dispersion, favoring population declines (Arntzen et al. 2017).

Brazilian subtropical wetlands are located in one of the most economically active regions of Brazil, with a colonization history of more than 200 years. Therefore, they are subjected to intense human pressure. These wetlands are considered regional biodiversity hotspots, providing shelter and refuge to aquatic organisms, but also to a large number of associated fauna (Maltchik et al. 2003). These wetlands encompass many floodplains of Atlantic Forest representing a transition zone between ecoregions (grassland/forest). In addition to their relevance due to the scarcity of studies, wetlands in Brazil's extreme south are subjected to a subtropical climate, which makes them systems with a particular configuration in climatic, faunistic and limnological terms. This study aimed to seek evidence in spatial and temporal scales of the effect of changes in the natural landscape of subtropical wetlands on the anuran diversity in Brazil's extreme south.

Materials and methods

Study area

Studied area is an interface between the biomes Pampa and Atlantic Forest in Brazil's extreme south, with a wide area of wetlands formed along the floodplain of the Sinos River (Maltchik et al. 2004). This floodplain contains a very large number of temporary ponds adjacent to urban areas that make up one of the zones with the highest ecological tension in southern Brazil. We monitored a group of small temporary ponds (up to 8 ha) used as breeding sites by anurans. We sampled 10 ponds distributed along the municipalities of Campo Bom, Novo Hamburgo, São Leopoldo, Sapucaia do Sul and Taquara, located at the southern limit of the Brazilian Atlantic Forest (29°48'35.99"S 51°9'23.25"W and 29°40'35.86"S 50°46'58.28"W). The region has a native vegetation cover composed of grasslands associated with shrubs and forest remnants with a predominance of *Mimosa bimucronata* (DC.) Kuntze, *Inga uruguensis* Hook. & Arn, *Salix humboldtiana* Willd and, in lower density, *Parapipitadenia rigida* (Bentham) Brenan and *Ficus organensis* (Miq.) Miq. (Rambo 2000). The topography of the sampling sites is plain and is inserted in the Sinos River Basin (Fig. 1). The region's climate is classified as Cfa, humid temperate marked by warm summers and annual precipitation between 11,162 and 309 mm (Maluf 2000).

Through the sampled area, there is variation in the preservation level of the wetlands in which the ponds are inserted. These changes include the emission of sewage, emission or accumulation of solid waste in the water bodies, presence of cattle, earthwork for housing and road construction and eventually sediment dredging for mining. At the same time, there are many relatively well-preserved wetlands. The characterization of these ponds as preserved followed the criteria of classification of southern Brazilian wetlands proposed by Maltchik et al. (2004) and detailed below. The selection of 10 sample ponds occurred in a paired way, each pair being composed of one pond in an area of high degree of preservation (reference ponds) and other in an area of high degree of degradation (altered ponds).

Sampling Design

A critical point in the study was the establishment of reasonable criteria for the classification of the wetlands and

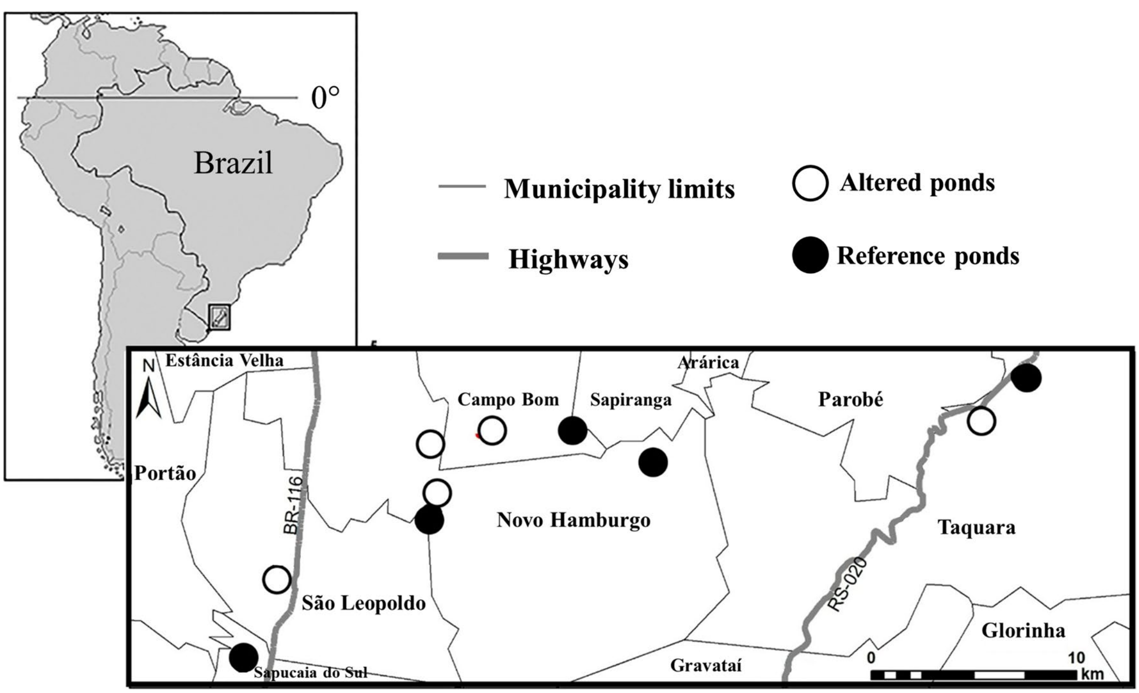

Fig. 1 Map showing the stretch of the Sinos River Basin in which samples occurred. Circles indicate reference ponds, with low degree of human interference; stars indicate altered ponds, with high degree of human intervention

their respective ponds (altered or reference). Although we initially sought an essentially objective criterion, we also adopted some subjective elements in our classification process (adapted from Maltchik et al. 2004). Our criteria can be summarized in the following manner:

1 – Altered ponds are located in areas with a population density estimated in at least 30 houses per km2, which was estimated visually in the field and using recent satellite images (Online Resource 1);
2 – Altered ponds are surrounded by roads with a moderate/high vehicle flow in urban vicinity areas;
3 – Altered ponds have visible accumulation of waste and open sewage.
4 – Reference ponds are located in areas with low population density (five houses per km2),
5 – Reference ponds are established in rural areas surrounded by local roads with a low vehicle flow (Online Resource 2).
6 – The region of reference ponds has a system of urban waste collection and basic sanitation and the reference wetlands are not used for the disposal of solid waste or domestic sewage.

Anuran Survey

Samplings occurred between June 2015 and May 2016. Pond monitoring for amphibian register was repeated monthly, with each pond sampled once every month. Considering that the anuran detection based on their calling activity vary according climatic variables (especially temperature and rainfall), we concentrated the monthly samplings within the smallest possible time window. Therefore, the time interval between the sampling of the first and the last pond did not exceed ten days within the same monthly sampling. We also randomized the order in which the ponds were sampled each month. To reduce the spatial dependence between samples sites, we selected ponds located at least 1.2 km from the next pond. This was the smallest possible distance that ensured that the ponds were not connected during the flood peak.

Anuran survey was performed by calling survey via an automated recording system (Heyer et al. 1994; Bridges and Dorcas 2000) using digital recorders (model Sony ICD-PX312/PX312F). In each reproductive site, we installed three recorders at a distance of about 200 m from each other to increase sound capture in the locality (Online Resource 3). The records extended for 13 continuous hours, beginning at 8:00 p.m. of one day and ending at 9:00 a.m. of the next day. The automated system guaranteed safer nocturnal sampling since most of altered ponds are located in areas with low social development and high rates of criminality.

The recording system units were attached to trees and placed near water bodies at a height of 1 m from the ground, allowing a homogenous sound capture. The audio files containing 13 h of record (equal to a monthly record of a reproductive site) were subdivided into stretches or samples. Each stretch was "extracted" from the total audio file by means of the software Audacity and had a duration of 5 min counted from each "clock hour". Thus, the stretches contemplated the record intervals from 8:00 p.m. to 8:05 p.m., from 9:00 p.m. to 09:05 p.m., from 10:00 p.m. to 10:05 p.m., and so on. As a result, the 13-hour record resulted in 14 audio samples, each with a duration of 5 min. The samples were heard using a Sony Headphone Mhz-300, allowing the identification of each amphibian species recorded in each sample. To identify the species, we used the sound guide for anuran amphibians of the Atlantic Forest (Haddad et al. 2005).

Evaluation of the Change in Land Cover

The evaluation of land cover occurred through the analysis of satellite images, which allowed the preparation of maps with the discrimination of different types of land cover. The types of land cover were defined initially through the observation of the landscape on the ground and later through the creation of classification units that could be differentiated from geoprocessing tools. The following categories of land cover were defined according to the dominant type of use in the image:

a) Urban area: area with predominance of residential and commercial buildings and paved roads;
b) Forest: area with predominance of forest formations, mostly native;
c) Wetlands: flooded land outside the riverbeds or floodplains;
d) Rivers and lakes: perennial water bodies;
e) Grasslands: formations with predominance of non-flooded grasses;

To prepare the maps, we used the satellite images LANDSAT 7 and 8, available at the Instituto Nacional de Pesquisas Espaciais (INPE). Specifically, we used a LANDSAT-7 image from 12/1999 and a LANDSAT-8 image from 12/2016. The digital processing of the satellite images was conducted by means of the software ArcGIS 10.3 using the tool of Interactive Supervised Classification. Supervised classification consists of making a manual selection of pixel groups that are representative of some observable feature in the image, such as the different pre-defined categories of land cover (see Oyamaguchi 2006). Based on the reflectance values of the Red-Green-Blue (RGB) channels of each previously selected pixel group, the software classified the whole image, distinguishing features that had pixel values similar to the selected groups. To evaluate the history of land cover around the different sampled ponds, the classification procedure was conducted in images from 1999 to 2016. For this comparison, we used the value of the difference between each category of land cover between the years. This subtraction value corresponds to a type of index, becoming a variable related to the current richness. The evaluation of land cover was conducted within a spatial scale that is larger than the known home range of some South American species (Tozetti and Toledo 2005; Oliveira et al. 2016). Thus, our buffer included an area extension of 500 m from the center of each pond, which could be crossed only through several generations of those populations. Therefore, from a spatial reference, this represents a reasonable area for the analysis of the influence of landscape on the species evaluated in this study.

Data Analysis

Richness, diversity, dominance and evenness of amphibians were represented by the total number of species (S), by the Shannon diversity index (H´), by the Dominance index (D) and by the Pielou's Evenness Index (J), respectively. Abundance was estimated through the number of calling males in each sample using the following abundance classes (e.g., Bertoluci and Rodrigues 2002; Ávila and Ferreira 2004; Ximenez and Tozetti 2015): (a) 1–4 calling individuals; (b) 5–9 calling individuals; (c) 10–20 calling individuals; d) > 20 calling individuals. In each sample (five-minute audio sample) we estimated the abundance of each species. For the total abundance of each species per pond, we used the maximum recorded abundance. The estimation of abundance for the classification of amphibians used the highest abundance class (maximum abundance) in the night for each species in each sampled pond. Thus, abundance data do not repeat or sum up any species.

To evaluate the efficiency of the amphibian samplings in each pond, we generated a mean species accumulation curve (collector's curve) by means of the program Estimates 8.2.0 (Colwell 2009), adjusted for 1,000 randomizations of the sample order. Species composition between altered and reference ponds was compared using the rarefaction method by means of the program Ecosim (Gotelli and Entsminger 2001), adjusted for 1,000 randomizations. We tested the Chao 1 estimator, which was chosen because it shows a fast stabilization and constancy of the extrapolated value, for 1,000 random repetitions of the samples, calculated using the program Estimates 8.2.0 (Colwell 2009). Variations in richness, diversity, dominance, evenness and mean abundance of anurans between reference and altered ponds were compared using a Student's t-test since all obtained data were normal according to the Shapiro-Wilk test. A paired t-test was applied for each class of land cover in order to make a temporal comparison of the studied areas. A Non-metric Multidimensional Scaling (NMDS) analysis based on species abundance was used to analyze the dissimilarities in anuran composition between reference and altered areas. This analysis was generated with Bray-Curtis dissimilarity data in two axes. Later, the variables of land cover were added to the ordination through the envfit function of the package Vegan (Oksanen et al. 2009) in the statistical program R ver. 3.2.4 (R Development Core Team 2012). In order to characterize the reference and altered areas according to the percental of land cover, we applied, on a comparison basis, Student's t-tests for each class of land cover. The only parameter of land cover that did not show normality was the Rivers and Lakes class. Therefore, this category was compared using the Mann-Whitney test. The wetlands class presented a marginally significant p-value (0.0599). Thus, we took the logarithms of the data to reduce the variances and highlight the differences. To test the normality of the obtained values, we used a Shapiro-Wilk test. A Principal Component Analysis (PCA) was used to determine how land cover changed in the sampled areas between the years 1999 and 2016. This analysis was generated with the percentual of each category of land cover in the two years. The analysis was performed in the program PAST 3.14 (Hammer et al. 2001).

Results

We recorded 23 anuran species belonging to six families: Hylidae (10), Bufonidae (2), Cycloramphidae (1), Leptodactylidae (8), Microhylidae (1) and Ranidae (1) (Table 1).

In reference ponds we registered 23 species, compared to 13 in altered ponds. The estimated richness obtained by the rarefaction method was also higher for reference ponds (between 23 and 44 species) than for altered ponds (between 13 and 16 species). The accumulation curve was built based on the record of anuran species and on visual and acoustic records in the studied area and did not show a tendency to stabilize, indicating that new species could still be recorded (Fig. 2).

The reference ponds showed higher richness ($t = 5.27$; $p < 0.05$) and higher mean value of Shannon diversity index ($t = 3.33$; $p < 0.05$) than altered ponds (Table 2). In addition, the mean dominance was higher in altered ponds than in reference ponds ($t = 2.59$; $p < 0.05$) (Table 2). The values of Evenness were not significantly different between the two pond types ($t = 1.935$; $p = 0.08$) (Table 2).

Ten species occurred exclusively in reference ponds: *Elachistocleis bicolor* (Guérin-Méneville, 1838), *Boana faber* (Wied-Neuwied, 1821), *Leptodactylus gracilis* (Duméril and Bibron, 1840), *Leptodactylus latinasus* Jiménez de la Espada, 1875, *Physalaemus lisei* Braun and Braun, 1977, *Pseudopaludicola falcipes* (Hensel, 1867), *Rhinella dorbignyi* (Duméril and Bibron, 1841), *Scinax tymbamirim* Nunes,

Table 1 Relative frequency (%) of records of anuran species in small temporary ponds at the Southern Brazilian Atlantic Forest between June 2015 and May 2016

Family/Species	Altered ponds	Reference ponds
Bufonidae		
Rhinella dorbignyi (Duméril & Bibron, 1841)	0	4.32
Rhinella icterica (Spix, 1824)	0.6	0.65
Cycloramphidae		
Odontophrynus americanus (Duméril & Bibron, 1841)	0.6	2.59
Hylidae		
Dendropsophus minutus (Peters, 1872)	3.9	4.54
Dendropsophus sanborni (Schmidt, 1944)	22.2	9.94
Boana faber (Wied-Neuwied, 1821)	0	4.54
Boana pulchella (Duméril and Bibron, 1841)	50.0	18.36
Pseudis minuta Günther, 1858	2.8	7.78
Pseudopaludicola falcipes (Hensel, 1867)	0	17.28
Scinax tymbamirim Nunes, Kwet, and Pombal, 2012	0	1.30
Scinax fuscovarius (A. Lutz, 1925)	0	2.16
Scinax granulatus (Peters, 1871)	0	1.08
Scinax perereca Pombal, Haddad & Kasahara, 1995	0.6	2.59
Scinax squalirostris (Lutz, 1925)	11.1	4.75
Leptodactylidae		
Leptodactylus fuscus (Schneider, 1799)	1.1	4.54
Leptodactylus gracilis (Duméril & Bibron, 1840)	0	0.65
Leptodactylus latinasus Jiménez de la Espada, 1875	0	0.22
Leptodactylus luctator (Hudson, 1892)	0.6	1.73
Physalaemus cuvieri Fitzinger, 1826	1.1	1.30
Physalaemus gracilis (Boulenger, 1883)	4.4	7.13
Physalaemus lisei Braun & Braun, 1977	0	1.08
Microhylidae		
Elachistocleis bicolor (Guérin-Méneville, 1838)	0	0.65
Ranidae		
Lithobates catesbeianus (Shaw, 1802)	1.1	0.86

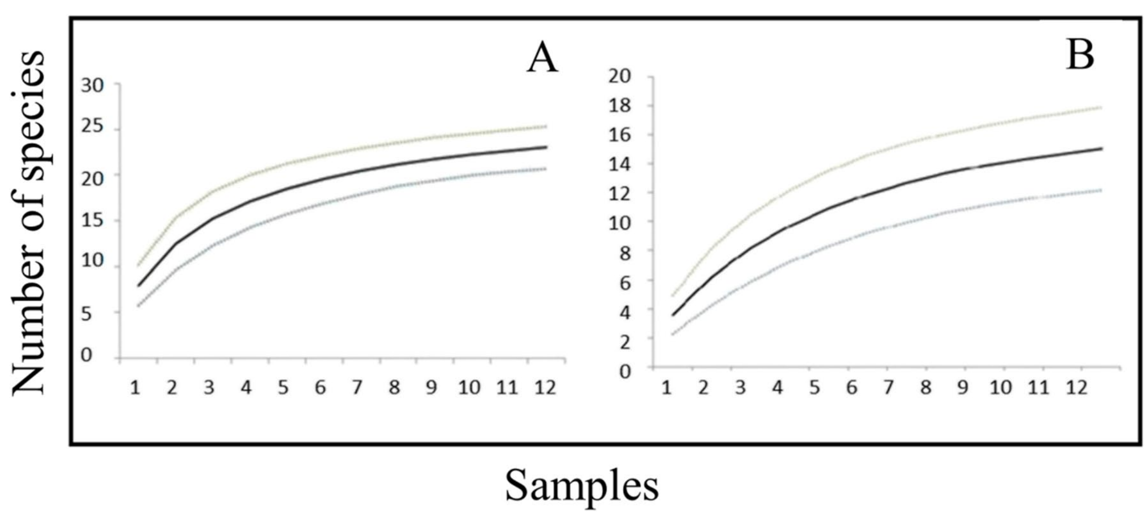

Fig. 2 Species accumulation curve of anurans recorded between June 2015 and May 2016 in subtropical small temporary ponds in the Southern Brazilian Atlantic Forest. The black line represents the mean value and the grey line represents the standard deviations. A = reference sampling units. B = altered sampling units

Table 2 Anuran diversity in small temporary ponds in the Southern Brazilian Atlantic Forest: Expected Richness by Jacknife1, Observed Richness, dominant species and observed dominance in the environments; richness and dominance observed by rarefaction in the reference ponds (confidence interval of 95%)

	Altered ponds	Reference ponds
Observed Richness	5.4	14
Expected Richness	15.5	23
Observed Dominance	0.4865	0.1753
Shannon0H	1.63	2.64
Evenness	0.62	0.84

Kwet, and Pombal, 2012, *Scinax fuscovarius* (Lutz, 1925) and *Scinax granulatus* (Peters, 1871) (Table 1). The species with the highest frequency of occurrence in reference ponds was *B. pulchella*, followed by *P. falcipes*, which were present in 18.36% and 17.28% of the samplings, respectively (Table 1). However, the frequency of occurrence of *B. pulchella* was higher in the altered ponds (present in 50% of the samplings), while *P. falcipes* was not present in any sampling of altered ponds (Table 1). Besides *B. pulchella*, *Dendropsophus sanborni* (Schmidt, 1944) was another species with a higher frequency of occurrence in altered ponds (present in 22.2% of the samplings).

The variation in the anuran species composition was represented by two axes in the ordination analysis (NMDS, stress: 0.08). The clear separation of the two polygons highlights the difference in the species composition between the ponds. Most species (*E. bicolor*, *L. latinasus*, *L. gracilis*, *P. falcipes*, *R. dorbignyi*, and *S. tymbamirim*) were found only in reference ponds. The most abundant species in reference ponds showed strong association with wetlands (*P. falcipes*) and grassland habitats (*D. sanborni*). The presence of forest habitat had little association with species composition. Some species showed a tendency to associate with urban areas, such as *Aquarana catesbeiana* (Shaw, 1802), *Physalaemus cuvieri* Fitzinger, 1826, and *S. granulatus*. The only species associated with altered reproductive sites was *B. pulchella*.

Characterization of the Changes of Natural Landscape (Land Cover)

Altered ponds are associated with a landscape with a higher percentage of urban area (32.68 ± 9%) than reference ponds (15.09 ± 5%) ($p = 0.0132$ and $t = -3.167$). Altered ponds also presented a smaller percentage of wetlands (1.23 ± 0.62%) than reference ponds (4.58 ± 3.4%), ($p = 0.0295$ and $t = 2.644$) (Fig. 3/Table 3). However, reference and altered ponds were not different regarding the percentage of rivers and lakes (mean for altered ponds = 8.51 ± 0.76%; reference = 0.84 ± 1.79%; $p = 0.204$ and $U = 6$), grasslands (mean for altered ponds = 39.44 ± 9; 57%; reference = 59.47 ± 22.47; $p = 0.104$ and $t = 1.833$) and forest (mean for altered ponds = 24.34 ± 13.78%, reference = 18.65 ± 13.76%; $p = 0.532$ and $t = -0.652$) (Fig. 3).

Regarding the difference in land cover between the years 1999 and 2016, we recorded significant variations in three categories: 1 - Urban area ($W = 0.932$ $p = 0.168$), 2 - Rivers and Lakes ($W = 0.88$ $p = 0.18$), and 3 - Wetlands ($W = 0.896$ $p = 0.035$) (Table 3). The temporal evaluation of the landscape showed that the urban area occupied on average 4.61% of the area surrounding reference ponds in 1999 and increased to 15.09% in 2016, which represents an expansion of 327.3%. In altered ponds, there was also an expansion, although smaller, of the urban area (143.6%). Wetlands decreased in all sampling sites. This reduction was higher in reference (31.3%) than in altered ponds (9.1%).

The PCA showed that the sampled reproductive sites had a higher association with wetlands and rivers and lakes (water bodies) in 1999 than in 2016. All sites had a tendency of higher association with urbanization in 2016 (Fig. 4). The analysis suggests that urbanization level and presence of grasslands and wetlands are the main factors allowing the separation of the sampling sites into reference and altered ponds (Fig. 4).

Discussion

We recorded 23 anuran species, similar number to that recorded by other studies carried out in wetlands in the south of South America (e.g. 23 spp., Peltzer et al. 2006; 10 spp., Moreira et al. 2010; 22 spp., Valério et al. 2016; 19 spp., Gonzalez Baffa-Trasci et al. 2020; 17 spp., Tozetti et al., 2023). We recorded almost 25% of the species that occur in wetlands of Brazil's extreme south (Borges-Martins et al. 2007; Machado and Maltchik 2007), suggesting a degree of tolerance of these amphibian species, despite the high degree of landscape change to which they are subjected. Corroborating our expectations, the reproductive sites in reference ponds had higher species richness than altered ponds. This pattern was reinforced by the diversity indices, which also reached the highest values in reference ponds. These indices also revealed lower dominance values in reference ponds, suggesting that species show less variation in their probability of establishing in these habitats than in altered areas. According to the richness estimator Chao 1, the number of expected species was close to the number of recorded species (Colwell and Coddington 1994), indicating a satisfactory efficiency of our sampling.

We recorded a dominance of Hylidae and Leptodactylidae in all ponds, which is also observed in several localities in Brazil and seems to be a common pattern in the Neotropical ecozone (Serafim et al. 2008; Trindade et al. 2010; Vilela

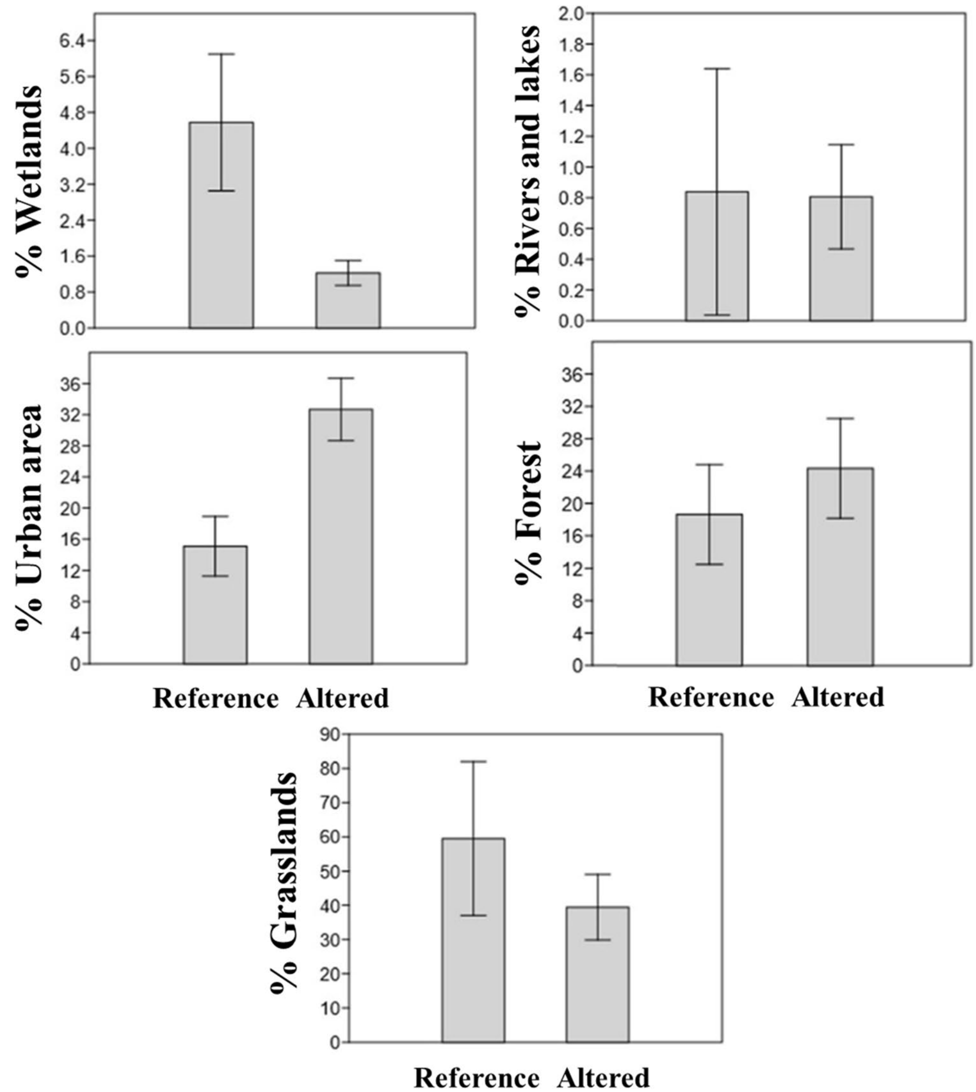

Fig. 3 Difference of land cover between reference and altered ponds for each classification of use, with their respective values of the statistical tests and p-values

et al. 2011; Maffei et al. 2011; Magalhães et al. 2013). Most species of these families are considered generalists regarding habitat, which could favor their establishment even in degraded areas (Brasileiro et al. 2005; Santos et al. 2016). According to Haddad et al. (2013), these families are able to colonize and survive in open areas, even with changes in the natural landscape, allowing their continuity in different matrix types. Some leptodactylids are also able to reproduce in environments with human alterations that lead to loss of plant biomass (Zina 2006). Despite the sampling effort, the species accumulation curves did not stabilize, indicating the potential to record new species in both reference and altered ponds. This fact is interesting, because it indicates the potential of the sampled habitats to harbor many species, even under human pressure. However, our results support the idea that the pattern of landscape change that occurred in the last decades in the study area has been suppressing the amphibian richness regionally. Our temporal evaluation regarding land cover indicated that, among the different processes of landscape change, the suppression of forest cover is one of the alterations with the highest damage to the maintenance of fauna. Removing forests generates microclimatic changes in water temperature, light intensity and humidity close to the soil surface (Halverson et al. 2003; Felix et al. 2004). Possibly, this change would affect more intensely species associated with forests than those associated with open areas or grasslands (Santos et al. 2016). Between 1999 and 2016, we observed an increase of 10.21% of the urban area, decrease of 2.08% in rivers and lakes and decrease of 11.18% in wetlands. There was no significant difference in the temporal variation of the categories grasslands and forest. A fact that calls attention is that the percentage of urban area increased significantly around all sampled ponds. At the same time, wetlands decreased in all locations. This result reveals the huge pressure that exists in the studied area. The fact that the negative changes (increase of urban area and

Table 3 Cover percentage of each category of land cover in each sampling unit and in the mean of reference and altered reproductive sites for the years 1999 and 2016

	UA 1999	UA 2016	RL 1999	RL 2016	GR 1999	GR 2016	FO 1999	FO 2016	WE 1999	WE 2016
R1	4.29	28.39	0.99	0.00	50.46	29.16	31.27	34.19	12.64	7.17
R2	4.79	17.66	8.21	4.04	38.01	43.09	36.55	31.79	12.08	1.68
R3	1.33	5.86	1.06	0.15	61.17	73.07	8.51	10.49	27.30	9.18
R4	6.50	10.98	0.00	0.00	73.38	83.11	5.13	2.67	14.58	1.79
R5	6.15	12.58	0.00	0.00	70.95	68.91	15.67	14.11	6.67	3.06
Mean R	4.61	15.09	2.05	0.84	58.80	59.47	19.43	18.65	14.65	4.58
A1	18.38	40.35	2.69	0.10	33.15	33.55	28.43	22.52	16.94	2.06
A2	14.66	19.19	6.96	1.58	19.34	29.84	47.64	47.51	11.39	0.57
A3	29.69	35.40	1.52	0.45	23.52	50.84	22.70	10.63	22.20	1.19
A4	33.82	40.19	5.56	1.67	24.14	34.36	28.03	21.39	8.00	0.72
A5	17.23	28.25	1.97	0.23	55.13	48.59	16.77	19.62	9.03	1.59
Mean A	22.75	32.68	3.74	8.51	31.06	39.44	28.71	24.34	13.51	1.23
Total Mean	13.68	23.89*	2.90	0.82*	44.93	49.45	24.07	21.49	14.08	2.90*

A altered pond, *R* reference pond, *UA* Urban Area, *RL* Rivers and Lakes, *GR* Grassland, *FO* Forest, *WE* Wetlands

reduction of wetlands) are more intense around reference ponds was expected since there is no space for expansion in the urban zone, nor wetlands to be suppressed around altered ponds. However, the results indicate that, although the reference ponds are visually in a better state of conservation, their surroundings have been suffering an intense process of landscape change. The combination of these two factors represents a loss of possible habitats and reproductive sites of anurans, which is inherent to the process of city expansion (Knutson et al. 2004). Besides habitat loss, the construction of roads, buildings and houses generate barriers for the dispersion of individuals and disturbs population dynamics (Gibbs 1998; Parris 2006). In a long-term study, Gagné and Fahrig (2010), showed that the cumulative effects of urbanization over time decreased the relative abundance of local species, which contributes to the depletion of the diversity of anurans. Our evaluation indicates that there is a continuous process of change of the reference ponds into altered ponds, which is alarming especially because some species were recorded exclusively in reference ponds (e.g., *E. bicolor*, *B. faber*, *L. gracilis*, *L. latinasus*, *P. lisei*, *P. falcipes*, *R. dorbignyi*, *S. tymbamirim* and *S. fuscovarius*).

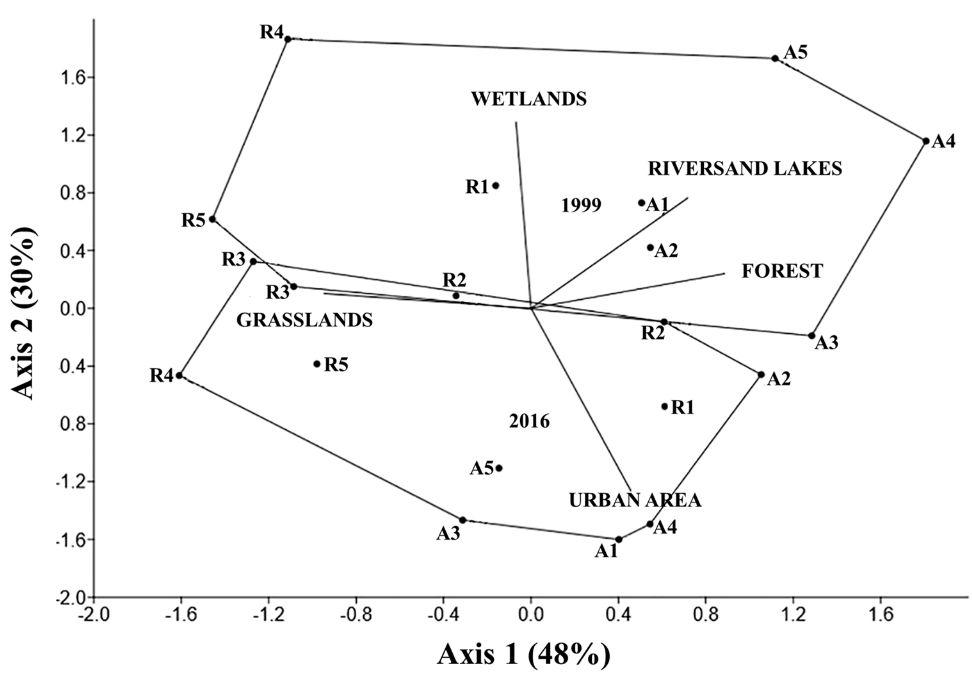

Fig. 4 PCA between the years 1999 and 2016 in relation to the categories of land cover and reproductive sites. A = altered pond; R = reference pond

With the exception of *P. lisei* and *S. tymbamirim*, all these species are considered relatively tolerant to the process of landscape change and have been recorded in urban and/or agricultural areas (Kwet et al. 2010; Maneyro and Carreira 2012). This reinforces the fact that even reference ponds are already suffering degradation. The presence of the bullfrog (*A. catesbeiana*) in the studied environments constitutes one more aggravating factor since this is an invasive species that competes for resources (Blaustein and Kiesecker 2002), may also influence the vocalization of amphibians (Medeiros et al. 2017), and preys on native anuran species (Hirai 2004; Govindarajulu et al. 2006). This species is widely recorded in southern Brazil (Both et al. 2011; Iop et al. 2011; Preuss 2017) and there are no coordinated actions for its management.

Three of the most common species in our study site in altered ponds, *B. pulchella*, *D. sanborni*, and *S. squalirostris*, were recorded in 11–50% of the samplings, with the remaining species being little frequent or absent. This suggests a tendency to the dominance of a small group of species when the natural landscape is altered. The three most abundant species are commonly found on the bordering vegetation in both permanent and temporary water bodies. These species are described as generalists regarding habitat that seem to tolerate a relatively high intensity of disturbances (Langone 1994; Kwet and Di-Bernardo 1999; Achaval and Olmos 2003; Condez et al. 2009). Despite the tolerance to disturbed habitats, the constant prevalence of individuals of *B. pulchella* possibly reflects the diversity of reproductive strategies used by this species, as well as the relatively high diversity of vegetation types that it uses (Haddad et al. 2013).

Many studies have highlighted a link of anurans to spatial heterogeneity and vegetation structure (Silva et al. 2012; Maragno et al. 2013). Knutson et al. (2004), showed a positive association between vegetation and wetlands with anurans. As the recorded species are mostly species of open and terrestrial habitats, the presence of wetlands and grasslands are more likely to affect their occurrence in the sampled sites. The direct impacts of human intervention, such as suppression of vegetation cover, wetland drainage, contamination of water and soil are real problems found in these environments (Marco et al. 1999). The remaining wetlands, although formed by small water bodies, make an important contribution for the maintenance of the regional biodiversity (Williams et al. 2003; Scheffer et al. 2006; Ruggiero et al. 2008; Gioria et al. 2010). However, as they are surrounded by areas with human impact, they are highly threatened, which intensifies the isolation of the associated biota (Boothby 2003). Landscapes that undergo changes for economic exploitation such as agriculture still maintain the potential of providing resources for some amphibian species (Knutson et al. 2004; this study). Although, water contamination by fertilizers and pesticides may be contributing, to the medium-term decline of several amphibian species (Cowman and Mazanti 2000; Linder and Grillitsch 2001; Agostini et al. 2013; Sanchez-Domene et al. 2018; Borges et al. 2019; Agostini et al. 2020). Our data reveal clear associations between the general pattern of the landscape and the general composition of anuran species. Currently, reference ponds present more than twice the number of wetlands and less than half the number of urban areas than altered ponds. This result suggests that the expansion of the urban area and the loss of wetlands is one of the main factors related to the depletion of anuran communities associated with the Southern Brazilian Atlantic Forest. By combining georeferencing tools to evaluate landscape change across recent years, we can choose priority areas for conservation and define management strategies to recover degraded areas.

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Author Contributions All authors contributed to the study conception and design. Material preparation and data collection were performed by Natália Oro and Camila Fernanda Moser. Analysis were performed by Alexandro Marques Tozetti, Marina Schmidt Dalzochio, Marcelo Zagonel de Oliveira, Arel Hadi and Jackson Fábio Preuss. The first draft of the manuscript was written by Natália Oro and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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