Effects of pine invasion on anurans assemblage in southern Brazil coastal ponds

Iberê F. Machado[1], Leonardo F.B. Moreira[1], Leonardo Maltchik[1,*]

Abstract. The destruction of wetlands due to afforestation areas is a common activity in temperate and subtropical regions in Southern America. The expansion of pine in the Coastal Plain of Southern Brazil is out of control and its impacts on aquatic biodiversity are little known. We tested the following hypotheses: the pine occurrence diminishes the anuran richness and abundance in ponds and it changes the anuran composition; the beta-diversity between pine and native grassland matrix ponds (natural ponds) is determined mainly by nestedness. Sampling was carried out from 2007 to 2009 in five ponds in pine invasion matrix and five ponds in native grassland matrix. The natural ponds showed a greater richness of tadpoles (10 species) than pine ponds (5 species). The mean richness of adults was higher in natural ponds than pine ponds throughout the entire study. The species composition was different between natural and pine ponds, for both adults and tadpoles. Comparing natural ponds with each other and the pine ponds with each other, our results showed that neither nestedness nor turnover was determinant for beta-diversity. However, when natural ponds were compared with pine ones, we found that the contribution of nestedness was higher for the anurans beta-diversity than turnover. Increases in the nestedness mechanism indicated that the pine occurrence results in species loss in Southern Brazil ponds. Since 90% of its wetland has been already affected, the removal of *Pinus* in the conservation areas in southern Brazil is important to minimize their impacts on aquatic biodiversity.

Keywords: anuran conservation, exotic species, hydroperiod, pine silviculture, wetlands.

Introduction

Scientific studies have shown in recent decades an accentuated decline in amphibian population, including the extinctions of species (Alford, Dixon and Pechmann, 2001; Collins and Storfer, 2003; Stuart et al., 2004). Habitat loss, fragmentation and alterations were the major cause of these disappearances (Blaustein and Wake, 1995; Global Amphibian Assessment, 2004). The indiscriminate use of pesticides and fertilizers in agro-ecosystems also has affected the living areas of amphibians (Relyea, 2005; Rohr et al., 2006). Many species of anurans have a highly permeable skin and their tadpoles are susceptible to changes in water chemistry (Vitt, Wilbur and Smith, 1990; Boone et al., 2007). The interspecific differences in life histories of amphibians, such as larval development time and the ability of locomotion, limit the dis-placement among aquatic habitats and their reproductive success (Babitt and Tanner, 2000).

Anurans are especially dependent on aquatic habitats for successful reproduction (Gerlanc and Kaufman, 2005). The loss of 50% of worldwide wetlands in the last 100 years (Shine and Klemm, 1999) has contributed to the disappearance of many amphibians species. Agriculture is one of the main human activities responsible for this decline (Czech and Parsons, 2002). In recent years, *Pinus* spp. monoculture had been introduced in many parts of the world as exotic species for commercial purposes. The destruction of wetlands due to afforestation areas (establishment of forests in areas where they did not occur previously) is a common activity in South America (Richardson, Williams and Hobbs, 1994; Nosetto, Jobbágy and Paruelo, 2005).

Pine silviculture has several effects on South American natural ecosystems – mainly in grasslands and dune areas due to strong invasiveness potential (Richardson, Williams and Hobbs, 1994; Bustamante and Simonetti, 2005). Such potential concerns the wetland biodiversity con-

1 - Laboratory of Ecology and Conservation of Aquatic Ecosystems, University of Vale do Rio dos Sinos, UNISINOS, Av. Unisinos, 950, CEP 93.022-000, São Leopoldo, RS, Brazil

*Corresponding author; e-mail: maltchik@unisinos.br

servation in southern Brazil, mainly when conservative data indicate that approximately 90% of the wetlands already disappeared in the last century (Maltchik et al., 2003). Southern Brazil wetlands are located in dunes and grasslands areas and the environmental conditions in this region – soil drainage, winds and pine cropping time – favour the dispersion and establishment of propagules of this exotic species (Richardson, Williams and Hobbs, 1994). However, the effects of pine invasion on the local biodiversity are little known (Rolon, Rocha and Maltchik, 2011).

The environmental impacts of planted forest have been studied worldwide (Wallace and Good, 1995; Richardson and Rejmánek, 2004). Afforestation of open habitats has consequences in the landscape and hydrologic characteristics. Forest implantation influences directly and indirectly the biota due to environmental changes, resource availability, shading, allelopathic effects and physicochemical conditions of the substrate (Richardson, 1998; Parris and Lindenmayer, 2004; Bustamante and Simonetti, 2005). The changes in the water balance are due to higher evapotranspiration rates of pine in relation to herbaceous species, which imply changes in the percentage of water available in the soil and drainage of aquatic systems (Lima, 1993; Gao, Zhang and Zhang, 2009). The conversion of grasslands into pine plantation may reduce the water yield from 20 to 80% (Zhang, Dawes and Walker, 2001; Nosetto, Jobbágy and Paruelo, 2005; Silveira and Alonso, 2009).

Environmental changes may alter species composition in areas invaded by exotic species (McKinney and Lockwood, 1999; Olden and Poff, 2003) both by species homogenization or establishment of new ones (McKinney and Lockwood, 1999; McKinney, 2004). The mechanism (turnover or nestedness) that will determine the beta-diversity (species composition dissimilarity) in invaded areas will indicate how the pine invasion will influence the native community structure (Baselga, 2010). While areas with beta-diversity generated by species

turnover suggest a high regional diversity (diversity γ), areas with beta-diversity generated by nestedness indicate a reduction in the number of species along an environmental gradient.

The expansion of pine (*Pinus elliottii* Engelm.) in the Coastal Plain of Southern Brazil is out of control and its impacts on aquatic biodiversity are poorly known. Assuming that the anuran abundance, richness and composition are strongly influenced by hydrological variation (Machado and Maltchik, 2010; Moreira et al., 2010) and by physico-chemical variables (Brodeman et al., 2003), we tested the following hypotheses: (1) the pine occurrence diminishes the anuran richness and abundance in ponds and changes the anuran composition; (2) the beta-diversity between pine and natural ponds is determined mainly by nestedness. Additionally, we analyzed the influence of water physicochemical characteristics on adult richness, abundance and composition.

Materials and methods

Study area

The study area is located in the buffer zone of Lagoa do Peixe National Park (LPNP), the only Ramsar site in southern Brazil (fig. 1). The LPNP (31°02′-31°48′S; 50°77′-51°15′W) has 34 400 ha, 62 km in length and an average width of 6 km. The Coastal Plain (State of Rio Grande do Sul) is one of the regions in Southern Brazil with the highest wetlands concentration (Maltchik et al., 2003). With a moist subtropical climate, the mean annual temperature is 17.5°C and ranges between 13°C and 24°C. The mean annual rainfall ranges between 1200 and 1500 mm (Tagliani, 1995).

Data collection

A total of seven sampling surveys were carried out in five ponds in pine invasion matrix – henceforth "pine ponds" – and five ponds in native grassland matrix – henceforth "natural ponds" (fig. 1). The ten studied ponds were randomly selected using the table of random numbers from a total of 25 pond sites (14 natural and 11 pine ponds) recognized from a topographic map inventory (1 : 50 000) of studied area. The samplings were carried out from November 2007 to October 2009, over the different year seasons (Nov/07, Feb/08, May/08, Aug/08, Oct/08, Feb/09, Aug/09 and Oct/09). All ponds had sizes ranging between 0.3 and 0.4 hectares and water depth no more than 0.4 m. The minimum distance between ponds was of approximately 500 m.
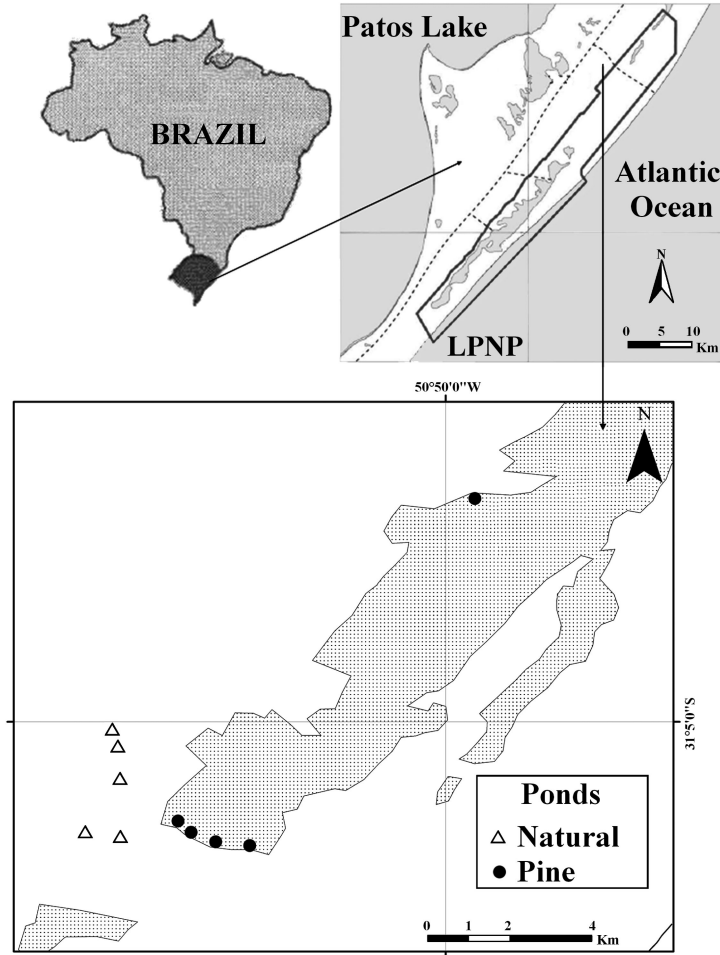
Figure 1. Localization of 10 studied ponds in the Lagoa do Peixe National Park (LPNP). The triangles represent natural ponds (n = 5) and the circles represent the pine ponds (n = 5).

The aggregate disposition of sampling units (ponds) was due to the low amount of ponds in study pine matrix and the low existence of pine matrices with similar characteristics in the study area (distribution, density and reproductive age). The pine distribution in the study matrix was irregular and the density was about 300 individuals per ha (ind.ha^{-1}). To calculated the density, we selected 3 quadrats of 10 m^2 (10 × 10 m) near the border of each pine ponds (n = 15) and all pine individuals were counted. All seedlings within each pine pond were counted (November 2007) and the density of seedlings within the ponds was about 2 individuals per m^2 (ind.m^{-2}). Pine was the unique arborous vegetation in study matrix (Diameter Breast Height was about 20 cm and height was about 15 m) and were distributed over all of the ponds borders (Perello et al., 2010). The ponds were surrounded by terrestrial vegetation and without surface water exchange (e.g. flooding).

Sampling methods

Richness and abundance of tadpoles were measured using the methodology proposed by Shaffer et al. (1994). The ponds were divided and numbered in small plots (2 m^2). Seven plots were randomized in each pond using a random numbers table before each sampling. Seven quadrats (50 × 50 cm) were sampled in the middle of each randomized plot per sampling occasion during the day. The quadrats were placed gently on the water surface, so as not to disturb any tadpoles on the pond bed. If any sign of tadpole movement resulting from disturbance was observed, the sampling site was abandoned and another site was randomized. All tadpoles inside the quadrat were removed using a dip net (10 cm wide). Sampling was finished in each quadrat only after 10 consecutive sweeps with no tadpole collection. The collected tadpoles were anesthetized with benzocaine, fixed in 5% formalin and deposited in the amphibian reference collection at UNISINOS. The tadpoles were iden-

tified following taxonomic keys specific to southern Brazil (Machado and Maltchik, 2007).

Richness and abundance of adult anurans were measured through visual and auditory search procedures (Crump and Scott, 1994). The amphibians were sampled through visual transects between sunset and 24:00. Six 10-min transects were sampled in each pond. This amounted to 60 min of sampling at each sampling site. We needed 3 consecutive days to sample the 10 ponds. Each transect had its starting point randomized and they were sequentially distributed to the length of ponds (20 meters each transect). On each sampling survey, the sequence of the pond visits was randomized using a table of random numbers. All the individuals located visually or acoustically were registered. Pilot studies have shown that 10 minutes was enough time for sampling the largest pond found in the area of the study. All individuals were identified according Cei (1980), Loebmann (2005) and Rosset (2008).

The physical and chemical characteristics of surface water were measured in three random points around the pond using HORIBA U-22 Water Quality Checker®. The parameters recorded were pH, dissolved oxygen (DO), water conductivity, oxidation-reduction potential (ORP), total dissolved solids (TDS) and turbidity. To describe the variability in water nutrient concentration over the study period (between seasonal periods), one sample of surface water (15 cm deep) was collected per sampling period in each pond using polyethylene bottles (500 mL). All samples were placed on ice in dark containers and taken to the laboratory, and immediately filtered upon return to the laboratory (Whatman® GF/F glass fiber filters, pore size 0.7 μm). Concentrations of nitrate (mg.L^{-1} NO$_3$-N) and soluble reactive phosphorus (mg.L^{-1} PO$_4$-P) were performed following the methodologies purposed by APHA (1989). The hydroperiod of each pond was measured by the number of sampling events with surface water (ranges between 1-7 events).

Data analyses

The richness of adults and tadpoles was represented by the number of species of adults and tadpoles observed in each wetland, respectively. The total and mean species richness and abundance were the cumulative and the mean number of the seven sampling events values, respectively. Adult and tadpole data were analyzed separately. Differences in species richness and abundance between natural and pine ponds over time were tested using Repeated Measures ANOVA. The analysis was performed using SYSTAT version 12. Levene's test verified the homogeneity of variance, and the Mauchy's sphericity test acknowledged the sphericity assumption. Assuming the compound symmetry (homogeneity of the variance-covariance matrix), no adjustment was made for the F test.

The differences of environmental variables measured (nitrate, soluble reactive phosphorus, percentage of organic matter, pH, DO, ORP, TDS, turbidity, conductivity and hydroperiod) between natural and pine ponds were compared by t-test. The analysis was carried out with the mean values collected over the two years. The ten environmental variables were reduced by Principal Component Analysis. For PCA ordination we used mean values of seven sampling and all variables were standardized by z-score. Then, the influence of environmental variables on total adult anuran richness and abundance were analyzed by multiple linear regression using scores of the two first axis of PCA ordination. The best fitted model was selected by Akaike Information Criterion (AIC – Akaike, 1974) and by selection method of both directions. The analyses were performed using R statistical program version 2.13 (R Development Core Team, 2011).

A Non-Metric Multidimensional Scaling was used to assess the variation of anuran species among pine and natural ponds. The NMDS is used to represent graphically the similarity in species composition in multiple dimensions. Only species that occurred in more than two samples were used. The analysis was performed with the Bray-Curtis dissimilarity using two axes by statistical package vegan (Oksanen et al., 2009) of R program, version 2.13 (R Development Core Team, 2011). Thus, the environmental variables were fit for the ordination by *envfit* function from the same statistical package. The significance of the relationship ($P < 0.05$) was obtained by 1000 permutations.

The spatial independence of the nine aggregate ponds was tested using PCNM analysis, by *quickPCNM* function of PCMN package (Legendre et al., 2010). As the global PCNM analysis had not detected significant spatial structure to adults ($P = 0.96$) and tadpoles ($P = 0.59$), it was not included in the statistical analysis. Besides, we did not observe variations in the six chemical variables measured of surface water (pH, dissolved oxygen, water conductivity, oxidation-reduction potential, total dissolved solids and turbidity) of four clumped pine ponds and the one pond located in the northern sector of the *Pinus* habitat. A Permutation Multivariate Analysis of Variance (PERMANOVA) was used to compare differences in species composition between natural and pine ponds. To assess differences in anuran species composition we used a Bray-Curtis dissimilarity matrix, and 9999 permutations to test model significance.

Beta-diversity and its partition in species turnover (Simpson dissimilarity index) and nestedness components were calculated according to Baselga (2010). Baselga (2010) suggested the beta-diversity partition (βsor – Sorensen pairwise dissimilarity) in two components: (1) turnover (βsim – Simpson pairwise dissimilarity) that indicates change in the composition due to different species assemblages between the pairs analysed; (2) nestedness (βnes – Nestedness-resultant dissimilarity) that indicates the loss of species between the pairs analysed, i.e., the species from poor areas are a sub-assemblage of richer areas. The analyses were carried out through the statistical program R, version 2.13 (R Development Core Team, 2011) using the "beta-pairwise" function provided by Baselga (2010). The values of beta-diversity from species turnover (βsim) and nestedness (βnes) components generated by the analyses of 10 studied ponds were separated into three groups: pairs of natural ponds (10 pairs); pairs of pine ponds (10 pairs); pairs of natural-pine ponds (25 pairs). The values of βsim and βnes were compared through t-test paired within each group previously determined. We used the one-tailed hypothesis as we intended to test whether the component determined by the species nestedness was higher or not than the component generated by turnover.

Results

We observed a total of 11 species of tadpoles over the study period (five in pine ponds and 10 in natural ponds) distributed in three families: Hylidae (six species), Leiuperidae (three species) and Leptodactylidae (two species). We captured 331 tadpole individuals in pine (78 individuals) and natural ponds (253 individuals). Hylidae represented the majority of individuals (46 and 128, pine and natural ponds respectively), followed by Leiuperidae (29 and 58, respectively) and Leptodactylidae (three and 67 individuals, respectively) (table 1).

We observed a total of 13 species of adult anurans distributed in four families, 13 species in natural ponds and 11 species in pine ponds. Hylidae was the most representative family (six species), followed by Leiuperidae (four species) and Leptodactylidae (two species). Bufonidae was represented only by one species (*Rhinella dorbignyi*) in natural ponds. We observed a total of 1157 individuals of adult anurans in natural (1036) and pine ponds (121). Hylidae also represented the majority of individuals in nat-

ural (733) and in pine ponds (50), followed by Leiuperidae (231 and 47, respectively) and Leptodactylidae (35 and 34, respectively) (table 1).

The maximum richness of tadpoles ranged from zero to nine species in natural ponds and between zero and four species in pine ponds. The tadpoles mean richness was higher in natural ponds than in pine ponds throughout the study period (Repeated Measures ANOVA $F_{1,8} = 131.056$, $P < 0.001$) (fig. 2a). The abundance of total tadpoles ranged from zero to 24 individuals in pine ponds and between zero and 28 in natural ponds. The abundance of total tadpoles was higher in natural ponds throughout the study period ($F_{1,8} = 8.198$, $P < 0.05$) (fig. 3a).

The maximum richness of adult anurans ranged from zero to eight species in pine ponds and between zero and 11 species in natural ponds during the study. The mean richness of adults varied between pine and natural ponds (Repeated Measures ANOVA $F_{1,8} = 171.79$, $P < 0.001$), being higher in natural ponds throughout the entire study ($F_{6,48} = 13.754$,

Table 1. Cumulative number of individuals for all anurans species recorded in natural and pine ponds in the National Park Lagoa do Peixe (LPNP) over the studied period (November 2007-2009).

Species	Natural ponds		Pine ponds	
	Adults	Tadpoles	Adults	Tadpoles
Bufonidae				
Rhinella dorbignyi	12	0	0	0
Hylidae				
Dendropsophus minutus	118	1	22	29
Dendropsophus sanborni	161	2	4	0
Hypsiboas pulchellus	211	79	6	6
Pseudis minuta	127	7	3	0
Scinax fuscovarius	22	10	2	0
Scinax squalirostris	129	29	0	11
Leiuperidae				
Physalaemus biligonigerus	10	4	4	0
Physalaemus gracilis	95	46	27	29
Physalaemus riograndensis	15	0	6	0
Pseudopaludicola falcipes	98	8	12	0
Leptodactylidae				
Leptodactylus gracilis	2	0	31	3
Leptodactylus latrans	36	67	4	0
Total richness	13	10	11	5
Total abundance	1036	253	121	78

$P < 0.001$) (fig. 2b). The total abundance of adult anurans ranged from zero to 32 in pine ponds and between zero and 67 individuals in natural ponds. The mean abundance of adults changed between natural and pine ponds ($F_{1,8} = 97.087$, $P < 0.001$) and over time ($F_{6,48} = 4318$, $P = 0.001$). The abundance of adults was higher in natural ponds throughout the entire study ($F_{6,48} = 4873$, $P < 0.001$), with higher values during the early spring and summer (Tukey $P < 0.05$) (fig. 3b).

Hydroperiod, TDS, DO and water conductivity were higher in natural ponds, while ORP was higher in pine ponds (t-test $P < 0.05$) (table 2).
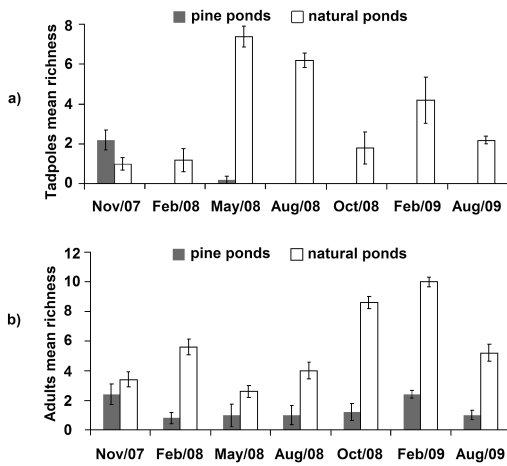
The environmental variables (n = 10) were reduced to two principal components, which explained 66.6% of the variation in the environmental characteristics. The first component represented 48.1% of the variation and was negatively related to hydroperiod, TDS, conductivity, DO, pH and percentage of organic matter and positively related to ORP. The second component explained 18.5% of the variation and it was positively related to nitrate and soluble reactive phosphorus concentration. The anuran richness was not affected by the first component nor to the second (Multiple linear regres-
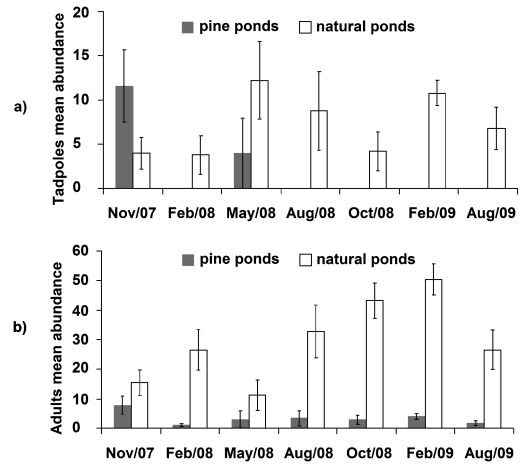

Figure 2. Anuran mean richness ($\pm$ SD) of natural and pine ponds in the National Park Lagoa do Peixe (LPNP) over the studied period (2007-2009). (a) tadpoles and (b) adults.

Figure 3. Anuran mean abundance ($\pm$ SD) of natural and pine ponds in the National Park Lagoa do Peixe (LPNP) over the studied period (2007-2009). (a) tadpoles and (b) adults.

Table 2. Mean values ($\pm$ standard deviation) scores of the PCA of environmental variables in natural and pine ponds in the National Park Lagoa do Peixe (LPNP) over the studied period (2007-2009). Asterisks indicate variables that are statistically significant ($P < 0.05$).

|	Natural wetlands mean ($\pm$ SD)	Pine wetlands mean ($\pm$ SD)	Scores	
			PCA1	PCA2
Conductivity (mS.cm^{-1})*	10.02 (4.14)	5.65 (1.09)	-0.80034	0.089549
DO (mg.L^{-1})*	10.29 (0.79)	8.78 (0.86)	-0.69638	-0.36209
ORP (mV)*	253.91 (20.19)	308.46 (11.75)	0.867141	0.129767
pH	6.63 (0.23)	6.28 (0.46)	-0.63509	-0.5946
TDS (g.L^{-1})*	0.06 (0.03)	0.04 (0.01)	-0.80846	0.076937
Turbidity (NTU)	61.64 (23.19)	79.46 (55.54)	0.167708	-0.35514
Nitrate (mg.L^{-1})	0.57 (0.23)	0.38 (0.05)	-0.49096	0.72293
Reactive soluble phosphorus (mg.L^{-1})	0.08 (0.05)	0.05 (0.01)	-0.54472	0.696972
Organic matter (%)	1.76 (0.86)	1.60 (1.01)	-0.53144	-0.31632
Hydroperiod (month)*	7.8 (0.45)	3.8 (1.92)	-0.88568	-0.01988
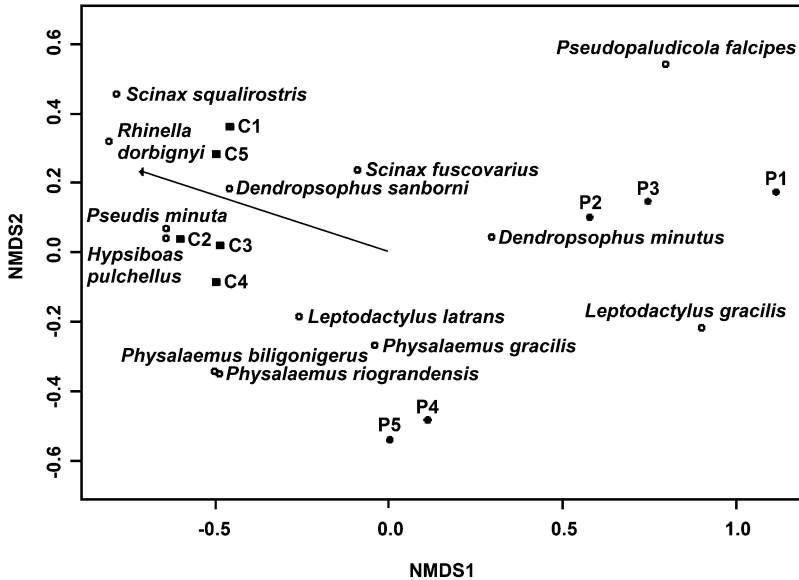
Figure 4. Ordination by non-metric multidimensional scaling (NMDS) of the occurrence of 13 species of adult anurans in the National Park Lagoa do Peixe (LPNP), over the studied period (2007-2009). C1-5 represents the five natural ponds and P1-5 represents the five pine ponds.

sion $r = 0.624$, $F_{2,7} = 0.058$, $P = 0.944$). The abundance of adult anurans was not affected by the first and second components of PCA ($r = 100.44$, $F_{2,7} = 0.203$, $P = 0.821$).

The axes generated by NMDS expressed a structure for the adult community, where the data were arranged according to the scores of the NMDS and the weighted average of the species. The value of stress (S) for the two axes of the NMDS (2-D) was equal to 1.2142^{-13}, indicating a significant accuracy to the representation of distances. The ordering produced by NMDS showed that the anuran composition in natural ponds was relatively distinct from that found in pine ponds (fig. 4). However, only the hydroperiod was correlated to anuran composition ($r^2 = 0.753$, $P < 0.01$). The gradient represented by the NMDS axes showed that the composition of adult anurans varied among natural and pine ponds (PERMANOVA $F_{1,9} = 11.29$, $P < 0.01$), with 10 species associated with natural ponds and two species associated with pine ponds.

The beta-diversity resulting from species nestedness among pairs of natural and pine ponds was similar to the beta-diversity resulting from turnover (natural-natural: t-test $t_9 = -0.129$, $P = 0.549$; pine-pine: $t_9 = -0.655$, $P = 0.735$, fig. 5). However, considering the pairs of natural-pine ponds, the value of beta-diversity determined by the nestedness differed significantly from the value resultant from the turnover (natural-pine: $t_9 = 2.143$, $P < 0.05$, fig. 5). Our results demonstrate that the beta-diversity found in pine ponds is a subset of the diversity of natural wetlands.

Discussion

The observed diversity in our study (13 species) comprised over 13% of richness known to southern Brazil and 82% of anurans richness of the studied region – Coastal Plain (Loebmann, 2005; Machado and Maltchik, 2007; Colombo et al., 2008). The diversity of tadpoles and anuran adults was lower in pine ponds throughout the entire study period (two years). Our results indicate that the lowest richness in pine ponds was independent of the temporal dynamics of amphibians. The presence of tadpoles
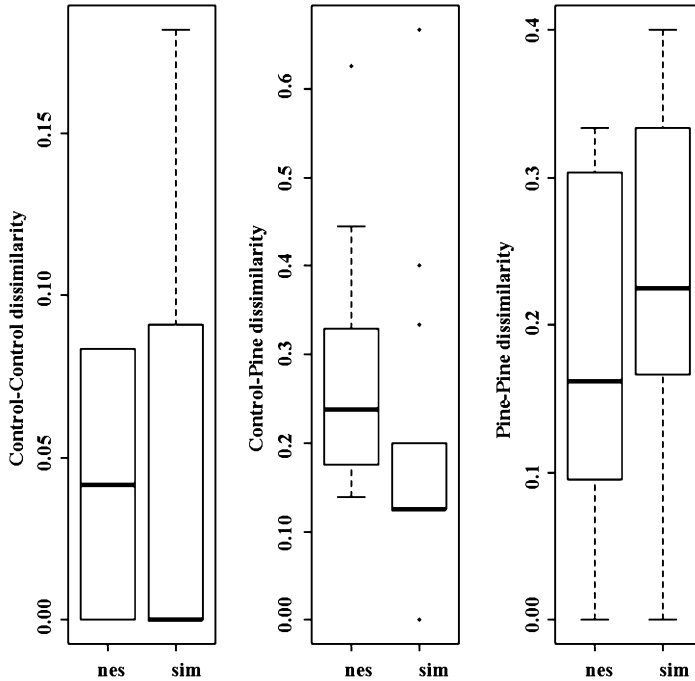
Figure 5. Relative contributions of species turnover (b-simpson) and nestedness (b-nestedness) for total beta-diversity, in the three groups of pairwise comparisons: natural-natural (n = 10 pairs), pine-pine (n = 10 pairs) and natural-pine (n = 25 pairs) in the National Park Lagoa do Peixe (LPNP) over the studied period (2007-2009). Nestedness (nes) and turnover (sim).

in pine ponds indicates that some amphibian species used the pine ponds as breeding sites, but always with a small number of individuals. Our results show that anurans are using pine ponds, including for reproduction, but with fewer species and lower abundance. Brode and Bury (1984) and Semlitsch and Bodie (2003) also found that amphibians use disturbed wetlands.

The process of habitat alteration changes the composition of biological communities (Patterson and Atmar, 2000) throughout species loss or to the replacement of species (McKinney, 2004). The beta-diversity variation among ecosystems reflects these two important ecological processes – the species nestedness and the species turnover (Baselga, 2010). Determining the contribution of these two processes in beta-diversity has important implications for species management. Our study tried to identify how pine invasion influences the anuran assemblage. If the variation of anuran composition between

studied ponds is generated by species turnover, we may predict that the pine occurrence provides an anuran composition different from natural ponds. However, if the beta-diversity is generated by nestedness, we may argue that the pine occurrence decreased the anuran richness, and the most affected species were those that were less tolerating environmental changes generated by the pine invasion. Comparing natural ponds with each other and the pine ponds with each other, respectively, our results show that neither nestedness nor turnover was determinant for beta-diversity. However, when natural ponds were compared with pine ones, we found that the contribution of nestedness was higher for the anurans beta-diversity than turnover. The increase in the nestedness component indicates that pine occurrence results in species loss in Southern Brazil ponds. Our result indicates that only part of the anuran assemblage uses the pine ponds in southern Brazil, and that the influence of *Pinus* is determining the loss of am-

phibian species in areas of pine occurrence. The reduction in richness and change in the species composition suggests that some species that inhabited these ecosystems were excluded, leaving only the most resistant to environmental changes induced by *Pinus* (Hazell et al., 2001; Guerry and Hunter, 2002). In our study, the most resistant species were *Leptodactylus gracilis* and *Dendropsophus minutus*.

The hydroperiod is considered a main environmental factor that influences anuran community in wetland systems (Semlitsch et al., 1996; Peltzer and Lajmanovich, 2004; Moreira et al., 2010). Changes in hydrological regimes (time in which wetlands remain with or without surface water) may be a direct consequence of *Pinus* occurrence in fields and dunes (Jobbágy and Jackson, 2004; Buytaert, Iñiguez and De Bièvre, 2007). Our results show that the hydroperiod was lower in ponds with *Pinus* than in natural ponds. The lower hydroperiod in these systems may have contributed to the lower amphibian richness and change in the composition, as environments with lower surface water can exclude species that depend on long periods of surface water for the development of their larvae (Babbitt, Baber and Tarr, 2003; Werner et al., 2007). For example, *Leptodactylus gracilis* and *Dendropsophus minutus* were better associated with pine ponds, due to the degree of independence of surface water for the development of tadpoles of *L. gracilis* (foam nests in underground chamber) and by the rapid development of the *D. minutus* tadpoles. However, the lowest richness and abundance, even in periods where all the ponds had surface water (natural and pine ponds) indicate that other environmental factors also affect the community of anuran species.

Amphibians are affected differently by many environmental variables according to their stage of development (Rogers and Chalcraft, 2008). Among the environmental variables evaluated in our study the total dissolved solids, dissolved oxygen, conductivity and redox potential varied among natural and pine ponds, but no relationship with richness, abundance or anuran composition was observed. Several studies investigated the influence of water chemistry on richness and anuran composition (Black, 1976; Wiggins, Mackay and Smith, 1980; Gerlanc and Kaufman, 2005), but the relationship between chemical variables and anuran community is not consensual (Vitt, Wilbur and Smith, 1990; Boone et al., 2007). Few studies showed positive relationship between water chemical variables and anuran community in southern Brazil (Both et al., 2009). Our results show that the physicochemical variables had less influence in anuran community than hydroperiod.

Our results suggest that the occurrence of *Pinus* has a negative impact on anuran assemblage, both in the larval as well as the adult stage. Pine ponds reduced the anuran richness and abundance and are changing the anuran composition. Among the species observed in pine ponds, only a subset was able to reproduce in these environments. The reduction in hydroperiod can be a reason for the decline of anuran species in these systems, however, shading and isolation provided by pines may also have contributed to this. However, our study was undertaken in a small section of southern Brazil and our results should be also verified in others areas within Brazil and the world. In terms of conservation and management, our results suggest the removal of *Pinus* existing in conservation units in southern Brazil as well as a proper management of pine plantations in order to minimize its expansions and impacts on the aquatic biodiversity. The wetland conservation and use of management practices that reduce human impacts on aquatic biodiversity are crucial in southern Brazil, since almost 90% of its wetlands have been lost.

References

Akaike, H. (1974): A new look at the statistical model
    identification. IEEE Trans. Automat. Contr. **19**: 716-
    723.

Alford, R.A., Dixon, P.M.E., Pechmann, J.H.K. (2001):
    Global amphibian population declines. Nature **412**: 499-
    500.

APHA (1989): Standard Methods for the Examination of
    Water and Wastewater. Washington, American Public
    Health Association.

Babbitt, K.J., Tanner, G.W. (2000): Use of temporary wet-
    lands by anurans in a hydrologically modified landscape.
    Wetlands **20**: 313-322.

Babbitt, K.J., Baber, M.J., Tarr, T.L. (2003): Patterns of lar-
    val amphibian distribution along a wetland hydroperiod
    gradient. Can. J. Zool. **81**: 1539-1552.

Baselga, A. (2010): Partitioning the turnover and nestedness
    components of beta-diversity. Global Ecol. Biogeogr.
    19: 134-143.

Black, J.H. (1976): Environmental fluctuations in central
    Oklahoma temporary ponds. Proc. Okla. Acad. Sci. **56**:
    1-8.

Blaustein, A.R., Wake, D.B. (1995): The puzzle of declining
    amphibian populations. Sci. Am. **272**: 52-57.

Boone, M.D., Semlitsch, R.D., Little, E.E., Doyle, M.C.
    (2007): Multiple stressors in amphibian communities:
    effects of chemical contamination, bullfrogs, and fish.
    Ecol. Appl. **17**: 291-301.

Both, C., Solé, M., Santos, T.G., Cechin, S.Z. (2009): The
    role of spatial and temporal descriptors for neotropical
    tadpole communities in southern Brazil. Hydrobiologia
    624: 125-138.

Brode, J.M., Bury, R.B. (1984): The importance of riparian
    systems to amphibians and reptiles. In: California Ri-
    parian Systems. Ecology, Conservation, and Productive
    Management, p. 30-36. Warner, R.E., Hendrix, K.M.,
    Eds, Berkeley, University of California Press.

Brodeman, R., Ogger, J., Bogard, T., Long, A.J., Pulver,
    R.A., Manchuso, K., Falk, D. (2003): Multivariate anal-
    yses of the influences of water chemistry and habitat
    parameters on the abundance of pond-breeding amphib-
    ians. J. Freshwater Ecol. **18**: 425-436.

Bustamante, R.O., Simonetti, J.A. (2005): Is *Pinus radiata*
    invading the native vegetation in Central Chile? Demo-
    graphic responses in a fragmented forest. Biol. Invasions
    7: 243-249.

Buytaert, W., Iñiguez, V., De Bièvre, B. (2007): The effects
    of afforestation and cultivation on water yield in the
    Andean páramo. For. Ecol. Manage. **251**: 22-30.

Cei, J.M. (1980): Amphibians of Argentina. Monograph 2.
    Monit. Zool. Ital. 609 pp.

Collins, J.P., Storfer, A. (2003): Global amphibian declines:
    sorting the hypotheses. Divers. Distrib. **9**: 89-98.

Colombo, P., Kindel, A., Vinciprova, G., Krause, L. (2008):
    Composição e ameaças à conservação dos anfíbios
    anuros do Parque Estadual de Itapeva, Município de Tor-
    res, Rio Grande do Sul, Brasil. Biota Neotropica **8**: 229-
    240.

Crump, M.L., Scott Jr., N.J. (1994): Standard techniques for
    inventory and monitoring: Visual Encounter Surveys. In:
    Measuring and Monitoring Biological Diversity. Stan-
    dard Methods for Amphibians, p. 84-92. Heyer, W.R.,
    Donnelly, M.A., McDiarmid, R.W., Hayek, L.C., Foster,
    M.S., Eds, Washington, Smithsonian Institution Press.

Czech, H.A., Parsons, K.C. (2002): Agricultural wetlands
    and waterbirds: a review. Waterbirds **25**: 56-65.

Gao, Z., Zhang, Z., Zhang, X. (2009): Responses of water
    yield to changes in vegetation at a temporal scale. Front.
    For. China **4**: 53-59.

Gerlanc, N.M., Kaufman, G.A. (2005): Habitat of origin
    and changes in water chemistry influence development
    of western chorus frogs. J. Herpetol. **39**: 254-265.

Global Amphibian Assessment [GAA] (2004): Summary of
    key findings. Available at http://www.globalamphibians.
    org. Accessed 10 Jun 2011.

Guerry, A.D., Hunter Jr., E. (2002): Amphibian distributions
    in a landscape of forest and agriculture: an examination
    of landscape composition and configuration. Conserv.
    Biol. **16**: 745-754.

Hazell, D., Cunningham, D.L., Mackey, B., Osborne, W.
    (2001): Use of farm dams as frog habitat in an Australian
    agricultural landscape: factors affecting species richness
    and distribution. Biol. Conserv. **102**: 155-169.

Jobbágy, E.G., Jackson, R.B. (2004): Groundwater use and
    salinization with grassland afforestation. Global Change
    Biol. **10**: 1299-1312.

Legendre, P., Borcard, D., Blanchet, G., Dray, S. (2010):
    PCNM: PCNM spatial eigenfunction and principal co-
    ordinate analyses. R package version 2.1. Available at
    http://R-Forge.R-project.org/projects/sedar/.

Lima, W.P. (1993): Impacto Ambiental do Eucalipto. São
    Paulo, EDUSP.

Loebmann, D. (2005): Guia Ilustrado: Os anfíbios da região
    costeira do extremo sul do Brasil. Pelotas, USEB.

Machado, I.F., Maltchik, L. (2007): Check-list da diversi-
    dade de anuros no Rio Grande do Sul (Brasil) e proposta
    de classificação para as formas larvais. Neotrop. Biol.
    Conserv. **2**: 101-116.

Machado, I.F., Maltchik, L. (2010): Can management prac-
    tices in rice fields contribute to amphibian conservation
    in southern Brazilian wetlands? Aquat. Conserv. **20**: 39-
    46.

Maltchik, L., Costa, E.S., Becker, C.G., Oliveira, A.E.
    (2003): Inventory of wetlands of Rio Grande do Sul
    (Brazil). Pesquisas: Botânica **53**: 89-100.

McKinney, M.L. (2004): Do exotics homogenize or dif-
    ferentiate communities? Roles of sampling and exotic
    species richness. Biol. Invasions **6**: 495-504.

McKinney, M.L., Lockwood, J.L. (1999): Biotic homoge-
    nization: A few winners replacing many losers in the
    next mass extinction. Trends Ecol. Evol. **14**: 450-453.

Moreira, L.F.B., Machado, I.F., Garcia, T.V., Maltchik, L. (2010): Factors influencing anuran distribution in coastal dune wetlands in southern Brazil. J. Nat. Hist. **44**: 1493-1507.

Nosetto, M.D., Jobbágy, E.G., Paruelo, J.M. (2005): Land-use change and water losses: the case of grassland afforestation across a soil textural gradient in central Argentina. Global Change Biol. **11**: 1101-1117.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H. (2009): Vegan: Community Ecology Package. R package version 1.15-2. Available at http://CRAN.R-project.org/package=vegan.

Olden, J.D., Poff, N.L. (2003): Toward a mechanistic understanding and prediction of biotic homogenization. Am. Nat. **162**: 442-460.

Parris, K.M., Lindenmayer, D.B. (2004): Evidence that creation of a *Pinus radiata* plantation in south-eastern Australia has reduced habitat for frogs. Acta Oecol. **25**: 93-101.

Patterson, B.D., Atmar, W. (2000): Analyzing species composition in fragments. In: Isolated Vertebrate Communities in the Tropics, p. 9-24. Rheinwald, G., Ed., Bonn.

Peltzer, M., Lajmanovich, R.C. (2004): Anuran tadpole assemblages in riparian areas of the Middle Paraná River, Argentina. Biol. Conserv. **13**: 1833-1842.

Perello, L.F.C., Guadagnin, D.L., Maltchik, L., Menezes, R.B., Stranz, A., Santos, J.E. (2010): Os desafios para a conservação do Parque Nacional da Lagoa do Peixe, RS. In: Faces da Polissemia da Paisagem – Ecologia, Planejamento e Percepção, Vol. III, p. 135-151. Santos, J.E., Zanin, E.M., Moschini, L.E., Eds, São Carlos, Rima.

R Development Core Team (2011): R: A Language and Environment for Statistical Computing. Austria, R Foundation for Statistical Computing.

Relyea, R.A. (2005): The lethal impact of Roundup on aquatic and terrestrial amphibians. Ecol. Appl. **15**: 1118-1124.

Richardson, D.M. (1998): Forestry trees as invasive aliens. Conserv. Biol. **12**: 18-26.

Richardson, D.M., Rejmánek, M. (2004): Conifers as invasive aliens: a global survey and predictive framework. Divers. Distrib. **10**: 321-331.

Richardson, D.M., Williams, P.A., Hobbs, R.J. (1994): Pine invasions in the Southern Hemisphere: determinants of spread and invadability. J. Biogeogr. **21**: 511-527.

Rogers, T.N., Chalcraft, D.R. (2008): Pond hydroperiod alters the effect of density dependent processes on larval anurans. Can. J. Fish. Aquat. Sci. **65**: 2761-2768.

Rohr, J.R., Keby, J., Sih, A. (2006): Community ecology theory as a framework for predicting contaminant effects. Trends Ecol. Evol. **21**: 606-613.

Rolon, A.S., Rocha, O., Maltchik, L. (2011): Does pine occurrence influence the macrophyte assemblage in Southern Brazil ponds? Hydrobiologia **675**: 157-165.

Rosset, S.D. (2008): New species of *Odontophrynus* Reinhardt and Lütken 1862 (Anura: Neobatrachia) from Brazil and Uruguay. J. Herpetol. **42**: 134-144.

Semlitsch, R.D., Bodie, J.R. (2003): Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. Conserv. Biol. **17**: 1219-1228.

Semlitsch, R.D., Scott, D.E., Pechmann, J.H.K., Gibbons, J.W. (1996): Structure and dynamics of an amphibian community: Evidence from a 16-year study of a natural pond. In: Longterm Studies of Vertebrate Communities, p. 217-248. Cody, M.L., Smallwood, J., Eds, New York, Academic Press.

Shaffer, H.B., Alford, R.A., Woodward, B.D., Richards, S.J., Altig, R.G., Gascon, C. (1994): Standard techniques for inventory and monitoring: quantitative sampling of amphibian larvae. In: Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians, p. 130-141. Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.C., Foster, M.S., Eds, Washington, Smithsonian Institution Press.

Shine, C., Klemm, C. (1999): Wetlands, Water and the Law: Using Law to Advance Wetland Conservation and Wise Use. Gland, IUCN.

Silveira, L., Alonso, J. (2009): Runoff modifications due to the conversion of natural grasslands to forests in a large basin in Uruguay. Hydrol. Process. **23**: 320-329.

Stuart, S., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fishman, D.L., Waller, R.W. (2004): Status and trends of amphibian declines and extinctions worldwide. Science **306**: 1783-1786.

Tagliani, P.R.A. (1995): Estratégia de Planificação Ambiental para o Sistema Ecológico da Restinga da Lagoa dos Patos – Planície Costeira do Rio Grande do Sul. Thesis, São Carlos, Federal University of São Carlos.

Vitt, J.P., Wilbur, H.M., Smith, D.C. (1990): Amphibians as harbingers of decay. BioScience **40**: 418.

Wallace, H.L., Good, J.E.G. (1995): Effects of afforestation on upland plant communities and implications for vegetation management. Forest Ecol. Manage. **79**: 29-46.

Werner, E.E., Skelly, D.K., Relyea, R.A., Yurewicz, K.L. (2007): Amphibian species richness across environmental gradients. Oikos **116**: 1697-1712.

Wiggins, G.B., Mackay, R.J., Smith, L.M. (1980): Evolutionary and ecological strategies of animals in annual temporary pools. Arch. Hydrobiol. (Suppl.) **58**: 97-206.

Zhang, L., Dawes, W.R., Walker, G.R. (2001): Response of mean annual evapotranspiration to vegetation changes at catchment scale. Water Resour. Res. **37**: 701-708.