

Assessing patterns of nestedness and co-occurrence in coastal pond anuran assemblages

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Abstract. Segregated species co-occurrence and nestedness are two ecological patterns used to measure assemblage structure. We investigated species co-occurrence and nestedness patterns in assemblages of tadpoles and adult anurans in 30 coastal ponds in southern Brazil. Ponds varied in hydroperiod and were classified as temporary or permanent. We explored whether co-occurrence or nestedness varied among ponds in each hydroperiod. Species co-occurrence patterns were analyzed using the C-score index and three null models. In order to quantify nestedness, we used the nestedness metric based on overlap and decreasing fill (NODF). We found seventeen anuran species; however, only 13 species were observed in breeding activity, and 11 species were observed as tadpoles. The co-occurrence and nestedness analyses showed that anuran assemblages exhibit non-random patterns that were generally contingent on the hydroperiod and pond area. Only species in non-breeding adult anurans assemblages showed significant segregation when randomizations were weighted by pond area and the pattern was similar among hydroperiods. Tadpole assemblages of permanent ponds showed an aggregated co-occurrence in weighted-fixed model. In temporary ponds, NODF showed that anuran assemblages were significantly nestedness in all three phases of life cycle. We obtained contrasting results depending on the hydroperiod. These results support the hypothesis that habitat nestedness, due to hydroperiod, might be an important factor structuring anuran assemblages along the different phases of their life cycle.

Keywords: breeding, hydroperiod, southern Brazil, tadpoles.

Introduction

Quantifying assemblage structure and revealing causal mechanisms is an important and growing area in conservation biology (Ricklefs, 2004). Segregated species co-occurrence and nestedness are two ecological patterns used to measure assemblage structure (Almeida-Neto et al., 2008; Heino, 2009; Simaiakis and Martínez-Morales, 2010). Co-occurrence patterns are usually attributed to competitive interactions or environmental filters. Additionally, these patterns may also be generated by habitat associations (Peres-Neto, Olden and Jackson, 2001), limited dispersal (Ulrich, 2004), and historical processes (Bloch, Higgins and Willing, 2007). Nestedness occurs in a set of assemblages where species at low diversity local-

ities are subsets of those at high diversity localities. There is a recognition that a nestedness pattern may be generated by differential dispersal (McAbendroth et al., 2005), passive sampling (Higgins, Willing and Strauss, 2006), differential habitat quality (Hylander et al., 2005), or habitat nesting (Wethered and Lawes, 2005).

Approaches to understand the mechanisms behind community structure have utilized null models to examine patterns occurring by chance or produced by interspecific interactions (Gotelli and McCabe, 2002). Null models based on randomization of species data usually preserve differences among species, such as species occurrence totals. However, some of these null models assume that species occurrences are random and independent, so they are null with respect to species interactions (Gotelli and McGill, 2006). Recent null model analyses have incorporated additional data weights, including site-level measures of habitat variability (Peres-Neto, Olden and Jackson, 2001), and species-level measures of population size (Gotelli and Ellison, 2002). The incorporation of additional

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data weights allow to analyze separately the effects of biotic interactions and environmental filters on assemblage structure (Fattorini, 2007; Heino, 2009; Both et al., 2011). In contrast to unweighted analyses, that treat sites and species with equal probabilities, such weightings adjust the probability of species occurrence at a site during randomizations (Heino, 2009).

Many studies have examined amphibian community structure through co-occurrence and nestedness analyses (Baber et al., 2004; Tockner et al., 2006; Watling, Gerow and Donnelly, 2009; Both et al., 2011). The anuran distribution patterns have been associated with area and isolation and their associated processes (extinction related with area and colonization) (Yiming, Niemelä and Dianmo, 1998; Tockner et al., 2006; Watling, Gerow and Donnelly, 2009), although there is no consensus on which mechanisms control the observed patterns in amphibians. Biotic interactions, such as predation and competition, also exert influences on anuran communities, either because the survival rate of tadpoles or the choice of breeding sites by adults (Dayton and Fitzgerald, 2001; Zina et al., 2007). A few studies have related nestedness and co-occurrence patterns with hydroperiod (Baber et al., 2004; Van Buskirk, 2005; Werner et al., 2007a). Anuran communities are often described as being organized along a hydroperiod gradient, ranging from ephemeral ponds to large permanent sites (Babbitt, 2005; Prado, Uetanabaro and Haddad, 2005). If this is true, the probability of a species to occur in a given area would also depend on pond hydroperiod and not on competition alone (Baldwin, Calhoun and deMaynadier, 2006; Karraker and Gibbs, 2009). In addition to hydroperiod, habitat diversity and aquatic plant occurrence also affect the anuran communities in pond systems (Kopp, Wachlevski and Eterovick, 2006; Moreira et al., 2010). Ponds with more complex habitats provide higher number of refuges for tadpoles to avoid predators (Rozas and Odum, 1988; Kopp, Wachlevski and Eterovick, 2006).

Adult anurans and tadpoles experience their environment in fundamentally different ways, so their community structure is influenced by different selective pressures (Duellman and Trueb, 1994). Tadpoles have little control of the habitat type where they develop (Alford, 1999). Variation in temporal patterns of reproduction results in tadpoles of different species experiencing different environmental conditions, especially in temporary ponds (Wells, 2007). Many species often breed in the same pond and avoid competition and predation pressure by segregating their calling sites and/or reproductive activity period (Kopp and Eterovick, 2006; Moreira et al., 2007). Adults can base their choices of ponds on factors others than the requirements of their larvae (Alford, 1999; Eterovick and Barros, 2003). As tadpoles and adult anurans show different ecological requirements, the occurrence patterns may change over the life cycle.

Our aim was to investigate species co-occurrence and nestedness patterns in anuran assemblages in coastal ponds. We also explored if occurrence patterns changed among the different phases of life cycle, or with pond area or hydroperiod. The patterns were examined in breeding adults, non-breeding adults, and tadpoles using data from a set of coastal ponds in southern Brazil. We looked for patterns of species occurrence following three hypotheses: 1) if species show more interspecific segregation among ponds than expected by chance, then competitive interactions are a possible underlying mechanism (Gotelli and McCabe, 2002); 2) if species are more aggregated than expected by chance, then similar responses to the environment are the most likely reason underlying the patterns (Sanders et al., 2007), 3) if species are not more segregated or aggregated than expected by chance, then patterns may comply with nestedness, thus suggesting different dispersal capabilities or tolerance quality as possible mechanisms (Hylander et al., 2005; McAbendroth et al., 2005).

Material and methods

Study area

The study area was the Lagoa do Peixe National Park (LPNP) and it is the only conservation unit protected by the Ramsar Convention in southern Brazil. The conservation unit has an area of 344 km² (31°02′-31°48′S; 50°77′-51°15′W), 62 km maximum length and a mean width of 6 km (Loebmann and Vieira, 2005a). The topography is basically flat, except for a line made up by the coastal dunes. The soil is made up of quartzose sands of marine origin. The humid subtropical climate implies a mean temperature range between 14.6°C in winter, and 22.2°C in summer, with a mean annual temperature of 17.5°C.

We selected thirty ponds from a topographic map inventory (1:50 000) of the Lagoa do Peixe National Park (fig. 1). All ponds had sizes ranging between 0.01 and 0.4 ha and water depth no more than 0.6 m. We sampled anuran amphibians in each one of the 30 ponds monthly for one year (from October 2007 to August 2008). We classified the ponds into permanent and temporary. Permanent were the ponds that maintained water in all samples, and temporary were the ponds that dried at least in one sample.

Anuran sampling

We sampled anurans through surveys in the breeding sites (Scott and Woodward, 1994). We sampled adults randomly between 7:00 PM and 0:00 AM for four consecutive nights. In each sampling, the entire margin of each pond was surveyed in 20 minutes. We registered all individuals located visually or acoustically. Pilot studies showed that 20 minutes were enough for sampling the largest studied pond. Enclosure sampling was used for tadpoles. We sampled seven quadrats (50 cm × 50 cm) randomly per sampling occasion in each pond system during the day. The quadrats were carefully placed on the water surface so as not to disturb any tadpoles on the pond bed. If any sign of tadpole movement resulting from disturbance were observed, the sampling site would be abandoned and another site would be chosen. We removed all tadpoles inside the quadrat 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 samples were taken to the laboratory for further processing and identification.

We classified anurans into three life stages: breeding adults, non-breeding adults, and tadpoles. Anuran calling male was used as an indicator of reproductive activity (Boquimani-Freitas et al., 2007). We considered non-

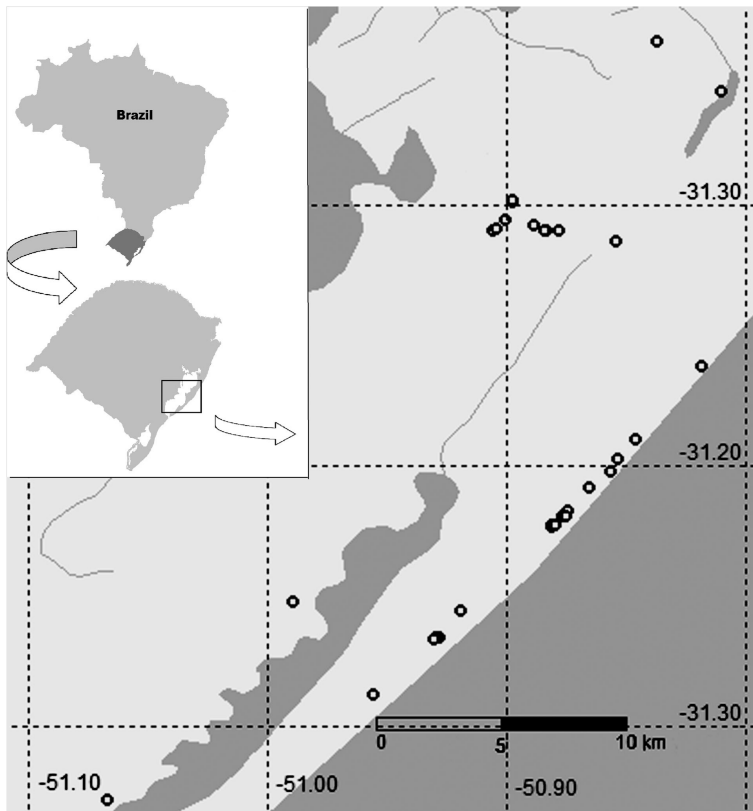


Figure 1. Study ponds in Lagoa do Peixe National Park, southern Brazil.

breeding adults only those found in samplings where no calling of the same species was heard in the pond.

Nestedness – co-occurrence analysis

Due to the hydroperiod effect on anuran community, we conducted the analysis in two ways: 1) we made no differentiation between hydroperiods; 2) we analyzed each hydroperiod group separately. A presence/absence matrix was constructed for each class with ponds in columns and species in rows. We calculated the C-score (Stone and Roberts, 1990) to measure the species co-occurrence. The C-score CS_{ij} for species pair ij is defined as: $CS_{ij} = (R_i - S)(R_j - S)$, where R_i is the row for total species i , and R_j is the row total for species j , and S is the number of ponds that contain both species. The C-score (hereafter CS) is calculated for all unique species pairs in the matrix and averaged (Stone and Roberts, 1990). If the communities were structured by competition, then the CS should be larger than expected by chance. That is, the larger the CS, the less the average pairwise species co-occurrence. Of the available measure of species co-occurrence, the CS has been shown to have the greatest statistical power for non-randomness detection (Gotelli, 2000).

The significance of the CS was tested using three null models. In the first null model (fixed-fixed), the row and column sums were fixed, i.e., each random pond contains the same number of species as the original pond and each species occurred in the same frequency as in the original assemblage (Gotelli and Ellison, 2002). This fixed-fixed model maintains the pattern of rich and poor sites in the randomized matrices. This is a traditional although very conservative null model (Gotelli, 2000). In the second null model (weighted-fixed), row weights were adjusted by the regional abundance of each species. Such a weighting should be done to account for species' abundance-related sampling effects on the observed patterns. This weighting assumes a positive occupancy-abundance relationship, which had previously been found for amphibians (Lemckert and Mahony, 2010). The weighted-fixed model is less conservative than fixed-fixed model and takes into account the importance of the sampling effects. Heino (2009) suggested that this model avoids the confusion with co-occurrence patterns. In the third null model (fixed-weighted), column weights were adjusted by pond area. Such a weighting is important, as the probability of a species to occur at a given site may depend on pond area and not competition alone. In this model, we incorporated the importance of the environmental descriptors in the community structure (Both et al., 2011).

For all null models, the random matrices were produced by shuffling the original matrix through repeated swapping of random submatrices. This algorithm is not prone to Type I or Type II errors (Gainsbury and Colli, 2003). In all analysis of co-occurrence for each species, 5000 random matrices were constructed and mean and standard deviation for the index values thus obtained were calculated. The statistical significance was then assessed by comparing the observed index value from the original matrix to the distributions of values derived from the random matrices. Species co-occurrence analysis and associated randomization were conducted using ECOSIM 7 (Gotelli and Entsminger, 2006).

For nestedness analysis, presence-absence matrices were first constructed where columns and rows were species and ponds, respectively. Nestedness by itself is not a metric, but a concept in which the species present in species-poor sites constitute proper subsets of those species present in species-rich sites (Almeida-Neto et al., 2008). We quantified the pattern of nestedness with the metric based on overlap and decreasing fill (NODF; Almeida-Neto et al., 2008), as recommended by Ulrich, Almeida-Neto and Gotelli (2009). NODF is a consistent metric to assess nestedness among sites only or among species only (Guimarães and Guimarães, 2006). NODF can assume values from 0 to 100, and higher values indicate more nested assemblages. Nestedness analysis requires an ordering of rows and/or columns of the incidence matrix according to some predefined criterion. So, we set incidence matrix according to two ways: order rows and columns by frequencies and an area-sorted matrix. There is a tendency for amphibian communities in ponds with small area and short hydroperiod to be nested subsets of those with the contrasting characteristics (Baber et al., 2004; Werner et al., 2007b). The different outcomes after sorting can be used to judge whether the pattern is linked to area-diversity relationships. To evaluate the significance of nestedness we used a null model with quasiswap algorithm and 999 simulations to validate the model significance (Miklos and Pósdani, 2004). The quasiswap method maintains both row and column frequencies. We performed the analyses using the Vegan 1.17 package in the R 2.11.1 environment (Oksanen et al., 2011).

Results

A total of 17 ponds were classified as permanent and 13 ponds as temporary. We identified 17 anuran species, but only 14 species were in calling activity. We observed eleven species in larval stages (table 1). Two species were found only in the temporary ponds (*Elachistocleis bicolor* and *Odontophrynus maisuma*), and two species were observed only in the permanent ponds (*Scinax berthae* and *Scinax fuscovarius*).

Calling species richness varied from 1 to 10 in temporary ponds and from 3 to 10 in permanent ponds. Non-breeding adults were found in all ponds, except for two ponds (one temporary and one permanent). Observed species richness ranged from 1 to 5 in temporary ponds and from 2 to 5 in the permanent ponds. Tadpoles were found in all ponds, except for two temporary ponds.

The CS patterns showed that species co-occurrence varied slightly among the different phases of life cycle (table 2). When all

Table 1. Species list and class classification of the 17 anuran species registered in 30 ponds, from October 2007 to August 2008, at Lagoa do Peixe National Park, Brazil.

Species	Permanent ponds			Temporary ponds		
	Non-breeding adults	Breeding adults	Tadpole	Non-breeding adults	Breeding adults	Tadpole
Bufonidae						
<i>Rhinella arenarum</i>	×			×		×
<i>Rhinella dorbignyi</i>	×	×	×	×	×	×
Cycloramphidae						
<i>Odontophrynus maisuma</i>				×	×	
Hylidae						
<i>Dendropsophus minutus</i>		×			×	×
<i>Dendropsophus sanborni</i>	×	×	×		×	×
<i>Hypsiboas pulchellus</i>	×	×	×	×	×	×
<i>Pseudis minuta</i>	×	×	×	×	×	×
<i>Scinax berthae</i>		×				
<i>Scinax fuscovarius</i>	×					
<i>Scinax squalirostris</i>		×	×		×	×
Leiuperidae						
<i>Physalaemus biligonigerus</i>	×		×	×		×
<i>Physalaemus gracilis</i>	×	×	×	×	×	×
<i>Physalaemus henselli</i>		×			×	
<i>Pseudopaludicola falcipes</i>	×	×		×	×	
Leptodactylidae						
<i>Leptodactylus gracilis</i>	×	×	×	×	×	×
<i>Leptodactylus latrans</i>	×	×	×	×	×	×
Microhylidae						
<i>Elachistocleis bicolor</i>					×	

ponds were jointly analyzed, no significant co-occurrence pattern was detected in the fixed-fixed and weighted-fixed models. Two analyses based on fixed-weighted algorithm were significant, with non-breeding adults and tadpoles showing segregation ($P = 0.001$ and $P = 0.027$, respectively). In temporary ponds only a single analysis based on the fixed-fixed algorithm showed non-breeding adults nearly significantly segregated ($P = 0.091$). One analysis based on fixed-weighted was significant, with non-breeding adults showing significant segregation ($P = 0.001$). In permanent ponds, a single analysis based on the weighted-fixed algorithm was significant, with tadpoles showing significant aggregation ($P = 0.037$). The breeding adults showed a nearly significant aggregation in the same algorithm ($P = 0.087$). By contrast, non-breeding adults were significantly segregated when species occurrence were fixed and ponds weighted by pond area ($P = 0.001$).

The nestedness patterns showed differences for frequency-sorted matrices. When all ponds were jointly analyzed, the anuran assemblages did not show significant nestedness (table 3). In temporary ponds, NODF showed that anuran assemblages were significantly nested in all phases of life cycle (table 3). Additionally, the degree of nestedness among ponds ($NODF_{rows}$) was lower than the degree of nestedness in species occupancy ($NODF_{columns}$) (table 3). Non-breeding adults was the class that showed higher differences in nestedness ($NODF_{rows} = 43$; $NODF_{columns} = 64.38$). In permanent ponds, no significant nestedness was observed in the three phases of life cycle. By contrast, when the statistic was evaluated with area-sorted matrix, the anuran assemblages did not show significant nestedness, in any hydroperiod. Moreover, the NODF values were lower for all phases of life cycle (table 3).

Table 2. Results of co-occurrence analyses for each class.

Hydroperiod	Class	Observed C	Simulated C		$P(obs \leq exp)$	$P(obs \geq exp)$	
			Mean	Variance			
Both	Fixed-fixed						
	Non-breeding adults	19.106	18.809	0.110	0.817	0.194	
	Breeding adults	7.890	7.585	0.107	0.843	0.166	
	Tadpoles	9.054	9.279	0.204	0.362	0.652	
	Weighted-fixed						
	Non-breeding adults	19.106	17.734	15.383	0.662	0.337	
	Breeding adults	7.890	9.891	4.684	0.177	0.822	
	Tadpoles	9.054	10.660	7.530	0.292	0.652	
	Fixed-weighted						
	Non-breeding adults	19.106	9.1706	3.015	1	0.001	
	Breeding adults	7.890	7.704	2.068	0.559	0.443	
	Tadpoles	9.054	6.707	1.3975	0.974	0.027	
	Temporary	Fixed-fixed					
		Non-breeding adults	5.889	5.694	0.018	0.931	0.091
		Breeding adults	1.744	1.607	0.012	0.894	0.118
Tadpoles		2.327	2.426	0.028	0.315	0.727	
Weighted-fixed							
Non-breeding adults		5.889	5.157	1.103	0.765	0.239	
Breeding adults		1.744	1.717	0.239	0.545	0.460	
Tadpoles		2.327	5.557	0.773	0.423	0.577	
Fixed-weighted							
Non-breeding adults		5.889	3.090	0.684	1	0.001	
Breeding adults		1.744	2.366	0.343	0.150	0.856	
Tadpoles		2.327	1.866	0.234	0.834	0.175	
Permanent		Fixed-fixed					
		Non-breeding adults	4.600	4.515	0.026	0.739	0.291
		Breeding adults	2.576	2.642	0.038	0.434	0.601
	Tadpoles	2.722	2.730	0.001	0.557	0.539	
	Weighted-fixed						
	Non-breeding adults	4.600	5.427	2.689	0.327	0.674	
	Breeding adults	2.576	4.014	1.216	0.087	0.914	
	Tadpoles	2.722	5.198	2.327	0.037	0.963	
	Fixed-weighted						
	Non-breeding adults	4.600	2.373	0.427	0.999	0.001	
	Breeding adults	2.576	2.478	0.416	0.577	0.432	
	Tadpoles	2.272	2.389	0.302	0.732	0.284	

Discussion

Adults and tadpoles exhibited non-random co-occurrence patterns that were generally contingent on the pond area and hydroperiod. Despite the significant segregation in some models, we observed significant nestedness patterns in the different phases of life cycle in temporary ponds. Studies that analyze co-occurrence and nestedness patterns in amphibians have observed divergent results according to the null

model used. Both et al. (2011) found random patterns when all community species were analyzed together and segregation patterns when the co-occurrence was analyzed by guild. Hecnar and M'Closkey (1997) found nestedness patterns at different spatial scales, but differences occurred among regions for some species. Baber et al. (2004) observed nestedness related to species richness, hydroperiod and pond area. In relation to the hydroperiod, the difference found in our results was not unexpected, since

Table 3. Measure of nestedness for the study ponds at Lagoa do Peixe National Park. N_{row} : nestedness among all rows (ponds), N_{col} : nested among all columns (species), Nestedness metric based on overlap and decreasing fill (NODF): total matrix nestedness, $\text{NODF}(q)$: nestedness of full model where presences are randomly assigned, but both row and column frequencies are maintained, $P(q)$: significance of NODF based on this null model.

Order	Hydroperiod	Matrix	N_{row}	N_{col}	NODF	$\text{NODF}(q)$	$P(q)$
Frequency	Both	Non-breeding adults	38.10	34.10	37.57	39.24	0.18
		Breeding adults	57.67	69.45	59.71	60.79	0.29
		Tadpoles	58.32	48.64	57.23	57.15	0.87
	Temporary	Non-breeding adults	36.86	38.15	37.33	34.82	0.05
		Breeding adults	43.00	64.38	53.69	51.94	0.04
		Tadpoles	41.32	47.37	43.82	41.37	0.01
	Permanent	Non-breeding adults	48.1	42.04	46.35	48.10	0.24
		Breeding adults	72.18	81.87	75.34	74.87	0.49
		Tadpoles	76.59	61.25	73.37	73.25	0.83
Pond area	Both	Non-breeding adults	18.83	23.94	19.5	19.05	0.65
		Breeding adults	47.81	39.23	46.32	46.53	0.77
		Tadpoles	39.09	27.01	35.07	35.32	0.78
	Temporary	Non-breeding adults	22.01	30	24.93	22.36	0.18
		Breeding adults	30.69	36.54	33.61	32.03	0.16
		Tadpoles	27.64	26.06	26.99	25.91	0.47
	Permanent	Non-breeding adults	47.52	32.78	44.44	44.52	0.89
		Breeding adults	63.77	49.54	59.12	58.96	0.88
		Tadpoles	47.52	32.77	44.43	44.47	0.94

the hydroperiod had been previously identified as an important factor for the anuran distribution in the coastal ponds (Moreira et al., 2010). In general, studies have found that hydroperiod is more important than pond area, and there is a turnover of species along the hydroperiod gradient (Van Buskirk, 2005; Werner et al., 2007a, 2000b).

Our results showed that breeding adults never showed any structure in coastal ponds, implying that their communities are random and are just coming together in random assemblages. Most of calling species were observed in both permanent and temporary ponds. In spite of being able to occupy different types of ponds, these species may have a restricted spatial distribution within them. Previous studies on adult anurans suggested that specific preferences for reproductive activities or development are more important in determining the spatial distributions of their assemblages than competitive pressures (Afonso and Eterovick, 2007). At NPLP, anuran assemblages are composed by a pool of species with prolonged calling activity patterns (i.e. *Dendropsophus sanborni*, *Hypsiboas pul-*

chellus, *Pseudis minuta*, and *Physalaemus gracilis*), and explosive breeders (*Rhinella arenarum*, *Rhinella dorbignyi*, *O. maisuma*, *E. bicolor*, *Leptodactylus latrans*). Explosive breeders call for few days and are often associated with heavy rain (Wells, 1977). Since some of amphibian species that use the ponds do so only in the reproductive season (Marsh and Trenham, 2001), it is not surprising that breeding adults would be selecting ponds by different ways, according to specific preferences. Studies on spatial and temporal distribution of anurans species often show segregation in calling sites and reproductive period (Kopp and Eterovick, 2006; Moreira et al., 2007). We observed non-random co-occurrence patterns only in the non-breeding adults, indicating that the temporal partitioning associated with reproduction is not responsible by occurrence patterns. In our study, the assemblages were sampled monthly, and data were pooled jointly in the analysis. We recognize that some species could use the same ponds at distinct times, and do not really co-occur.

In permanent ponds, tadpoles showed an aggregated structure, however, this pattern was de-

tectable only in weighted-fixed analyses. This aggregation was likely due to the fact that species' responses reflect that environment act as similar filter for species. Previous studies on amphibian assemblages suggest that the susceptibility to fish predation may be an important factor that influences the amphibian richness and distribution (Hecnar and M'Closkey, 1997; Babbitt, Baber and Tarr, 2003; Werner et al., 2007a). Amphibian species that can breed in fish inhabited ponds have toxic/unpalatable tadpoles (i.e. bufonids), or tadpoles with anti predatory behavior (i.e. leiuperids). However, the effectiveness of these defensive strategies is context dependent, varying with predator type and co-occurring species (Nomura et al., 2011). Predatory fishes, including thin dogfish (*Oligosarcus* spp.), catfish (*Rhamdia* spp.), and wolf fish (*Hoplias* spp.) occurred in nine of the 17 permanent ponds (Loebmann and Vieira, 2005b). Compared with temporary ponds, permanent ponds were deeper, larger, and typically had higher diversity of plants and a higher proportion of emergent and submerged vegetation in the study area (Rolon et al., 2008). Ponds with more complex habitats provide higher number of refuges for tadpoles to avoid predators (Babbitt, Baber and Tarr, 2003; Kopp, Wachlevski and Eterovick, 2006). Thus, this pattern of aggregation can be due to the accumulation of species with traits that can achieve positive fitness along the predation pressures. The aggregation restricted to analyses weighted by specie's abundance supported the hypothesis of interaction with fish, since population densities of tadpoles are much lower with fish than in their absence (Van Buskirk, 2005; Werner et al., 2007b).

By contrast, adults did not show an aggregated structure, and when the null models were adjusted by pond area, non-breeding adults showed significant segregation in all the analyzed hydroperiods. The effects of pond area on amphibian species are controversial (Babbitt and Tanner, 2000; Ficetola and De Bernardi, 2004), although some researchers have found

substantial evidences of relationships (Werner et al., 2007a). The evidences for the species-area relationship may be either an effect of area per se or other attributes associated with area, as habitat diversity. Long-term survey data on amphibian assemblages suggest that community assembly is a function of local and regional factors (Van Buskirk, 2005; Werner et al., 2007a). This relationship supports the hypothesis that species have different niches, and that more complex habitats can provide more niche opportunities. At NPLP, open areas, such as sand dunes and grasslands, are common, and they may affect differentially the species dispersion. Rates of evaporative water loss may be an important factor that determines the dispersion capacity in terrestrial systems. Species more resistant to desiccation may be more widely distributed in patchy landscapes, than species with higher rates of water loss. Although we did not measure the degree of isolation among ponds, the segregation pattern adjusted by pond area can be interpreted as the effect of dispersal limitation proportional to rates of evaporative water loss.

The significant nestedness observed only in the temporary ponds suggests that hydroperiod gradient is important because of developmental constraints. Congruent with the hypothesis of an independent effect of hydroperiod, no assemblages were significantly nested regardless of ordering by pond area. Although anurans usually show plasticity during larval development (Rowe and Dunson, 1995; Kwet, 2001), each species needs a minimum hydroperiod to complete development and metamorphosis. Besides, hydroperiod is an environmental gradient which directly or indirectly influences other important environmental factors to the structure of amphibian community such as temperature, water chemistry, productivity and structural characteristics of the pond (Wellborn, Skelly and Werner, 1996; Snoodgrass et al., 2000; Both et al., 2009). As in the case of predation by fish, this pattern of occurrence may arise due to the accumulation of species with traits that can achieve

positive fitness along the hydroperiod gradient. However, unlike pond area and fish presence at permanent ponds, individual temporary ponds can vary widely in annual hydroperiod. Long-term survey data showed a clear effect of annual variation in hydroperiod on species richness and turnover in ponds (Werner et al., 2007a, 2007b). Despite our study have been performed in a relatively short time period, the results showed a higher contribution of species occupancy for nestedness patterns than differences in species composition among ponds. This may be associated to extinctions due to weather variation affecting hydroperiod. Again, this factor should interact with species dispersal ability and connectivity of the ponds, and how often these extinctions occur.

Our results indicated that anuran occurrence showed contrasting patterns depending on the null model used. Corroborating other studies (Jenkins, 2006; Meyer and Kalko, 2008; Both et al., 2011), the analyses were sensitive to weighting factors. So, co-occurrence should incorporate weights for important factors likely to contribute to the observed patterns, such as pond area. However, incidence matrices ordered by pond area did not show nestedness in any hydroperiod. As we were not able to determine differences in the hydroperiod of each pond, we placed ponds into hydroperiod categories. A more detailed examination of hydroperiod/pond area patterns may be instructive. In organisms with biphasic life cycle, distribution patterns may change according to ontogenetic development, and the mechanisms associated with these patterns appear to be related to the environmental heterogeneity generated by hydroperiod fluctuations.

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