Anuran amphibians dynamics in an intermittent pond in southern Brazil

Dinâmica de anfíbios anuros em uma lagoa intermitente no sul do Brasil

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Abstract: Small wetlands are particularly important habitats for amphibians due to their close dependence on these habitats for their critical life-story functions. The constant degradation of wetland systems has been considered as one of the main causes for the declining of the anuran population observed around the world, including in southern Brazil. The objective of this study was to analyze the richness, abundance, and composition of the amphibian assemblage in a small pond associated to a floodplain system in a Neotropical region in Southern Brazil over a year. A total of 19 amphibian samplings were carried out over a year (from September 2004 to August 2005), and the survey was made in five transects (30 x 2 m) distributed at random along the studied pond. A total of 17 anuran species distributed in six families (Bufonidae, Cycloramphidae, Hylidae, Leiuperidae, Leptodactylidae, and Microhylidae) were observed. The Hylidae family represented 35.3% of the total species, and 22.0% of the total individuals observed over the studied period. Rhinella fernandezae was the most abundant species, representing 24.7% of the all collected individuals. The amphibian richness and abundance did not show any variation during the study period and between phases with and without superface water. However, the anuran composition changed between periods with surface water and without surface water. Air temperature and rainfall did not influence the amphibian richness and abundance. The high diversity and abundance of the anuran observed (17 species and 332 individuals) point to the need to include small wetlands in the biodiversity conservation programs in Southern Brazil, mainly when 90% have already disappeared due to anthropic activities. But these results should serve as hypotheses for long-term investigations in a larger area.

Keywords: anurans, seasonal change, drought, small wetlands.

Resumo: Áreas úmidas pequenas são hábitats importantes para os anfíbios devido a sua dependência desses locais para estágios críticos da história de vida. A degradação constante das áreas úmidas ao redor do mundo tem sido considerada uma das principais causas do declínio de populações de anfíbios, inclusive no sul do Brasil. O objetivo deste estudo foi analisar a riqueza, a abundância e a composição de uma assembléia de anfíbios em uma pequena lagoa associada a uma planície de inundação em uma região Neotropical, do sul do Brasil durante um ano. Foram realizadas 19 coletas ao longo de um ano (setembro de 2004 a agosto de 2005) e as amostragens foram feitas em cinco transectos (30 x 2 m) distribuídos aleatoriamente ao longo da lagoa estudada. Foram observadas 17 espécies de anuros distribuídos em seis famílias (Bufonidae, Cycloramphidae, Hylidae, Leiuperidae, Leptodactylidae e Microhylidae). A família Hylidae representou 35,3% do total de espécies e 22,0% do total de indivíduos observados. Rhinella fernandezae foi a espécie mais abundante, representando 24,7% de todos os indivíduos encontrados. A riqueza e a abundância de anfíbios não variou durante o período de estudo e entre as fases com e sem água superficial. No entanto, a composição de anuros variou entre as fases com e sem água superficial. A temperatura do ar e a precipitação não influenciaram a riqueza e a abundância de anfíbios. A alta diversidade e a abundância de anuros observados na lagoa (17 espécies e 332 indivíduos) enfatiza a necessidade de incluir as áreas úmidas pequenas em programas de conservação de biodiversidade no sul do Brasil, principalmente quando 90% das áreas úmidas dessa região já foram perdidas por atividades antropogênicas. Entretanto estes resultados deveriam servir como hipóteses a serem testadas para períodos mais longos e em escalas espaciais maiores.

Palavras-chaves: anuros, mudanças sazonais, seca, áreas úmidas pequenas.

1. Introduction

The understanding of patterns of abundance and distribution of organisms is one of the main goals in ecology. Diversity and composition of anuran assemblages have been attributed to many factors, such as rainfall (Toledo et al., 2003; Gottsberger and Gruber, 2004), hydrological cycles (Arzabe et al., 1998; Babbitt and Tanner, 2000), altitude (Fauth et al., 1989), habitat duration (Eterovick and Fernandes 2002), and predation (Fauth and Resetarits, 1991; Eterovick and Sazima, 2000). Temperature influences water balance, calling, metamorphosis, development, and immune response (Rome et al., 1992). Rainfall initiates reproduction for most pond-breeding anurans (Eterovick and Sazima, 2000; Prado et al., 2005; Kopp and Eterovick, 2006). Hydroperiod, the time a temporary pond holds surface water, is an important factor influencing the timing of metamorphosis (Parris, 2000), recruitment in amphibian populations, and structuring communities (Babbitt and Tanner, 2000; Ryan and Wine, 2001). Anurans are especially dependent on water and/or atmospheric humidity, because they are vulnerable to desiccation, which can affect their distribution and habitat use (Duellman and Trueb, 1994). A number of studies on anuran assemblages have demonstrated a temporal segregation, mainly associated to the breeding seasons (Bertoluci and Rodrigues, 2002; Gottsberger and Gruber, 2004).

Small wetlands are particularly important habitats for the anurofauna due to their close dependence on these habitats for their critical life-story functions (e.g., Barinaga, 1990; Borchelt, 1990). Some anuran species only breed in small wetlands (Dodd ,1997), however, the importance of these systems for the anuran assemblage is largely unstudied in Southern Brazil. Such gap becomes absolutely worrying considering that approximately 72% of inventoried wetlands in Southern Brazil are intermittent and smaller than 1 km² (Maltchik, 2003; Rolon and Maltchik, 2006). Besides, approximately 11.23% of the total amphibian fauna in Brazil, country with the greatest amphibian diversity in the world, were identified in Southern Brazil (Machado and Maltchik, 2007; SBH, 2008).

The objective of this study was to analyze the richness, abundance, and composition of the amphibian assemblage in an intermittent pond in Southern Brazil over a year. In this survey, we analyzed the effects of air temperature, rainfall, and lacking of surface water on the amphibian assemblage. Our approach was an exploratory analysis to infer the importance of intermittent ponds for the maintenance of biodiversity across wetland landscape for further hypothesis testing.

2. Material and Methods

2.1 Study area

This study was developed in an intermittent pond associated to a floodplain system in the lower course of the Sinos River, in Southern Brazil (Rio Grande do Sul - RS). The annual precipitation in the Sinos River basin (~4,000 km²) ranges from 1,200 to 2,000 mm, and it is well distributed along the year. The mean temperature varies between 15 and 18 °C. The minimum temperature is lower than 10 °C in winter (May to September), and higher than 32 °C in summer (December to March) (Radambrasil, 1986). The studied floodplain has approximately 30 ha, and it shows several permanent and intermittent ponds. During the flood events, water penetrates into the floodplain system in different stream reaches.

The studied site is an intermittent pond (29° 42' 16" S and 50° 59' 21" W, 21 m elevation), fed by water from rainfall, runoff and flood events from the Sinos River. The pond has a surface area of approximately 950 m², and it is approximately 300 m distant from the main channel of the Sinos River. The mean water depth is approximately 40 cm; however, during the flood events, the water depth can reach 200 cm in all floodplain system. The substratum of the studied pond consists of silt and organic debris. Extended beds of *Ludwigia pepolides* cover 40% of the water surface of the studied pond. Two different types of vegetation prevail in the riparian ecosystem: 60% of native woodland (mainly *Mimosa bimucronata*) and 40% of grasslands.

2.2 Amphibian sampling

A total of 19 amphibian surveys were carried out over a year (from September 2004 to August 2005) in the studied pond. The survey was made in five transects distributed at random within the pond (30 x 2 m). Transects were set randomly in each survey, and the sampling efforts for each transect lasted 20 minutes, totalizing 1 hour 40 minutes of sampling per survey. All surveys were made from 6:00 to 12:00 PM because all frog species are much more active in the night than during the day at the study site. The temporal sequence of transects surveyed each night was randomized, and the same team assessed all transects. Individuals were located visually or acoustically over the transects (Crump and Scott, 1994). Voucher specimens were deposited at the reference collection of Laboratório de Ecologia e Conservação de Ecossistemas Aquáticos (LECEA) - UNISINOS.

Air temperature, water depth, and area were measured "in situ" in the beginning of each survey. The air temperature was measured using an accurate mercury thermometer of 0.1 °C; the water depth was measured with a PVC tube graduated in centimeters, and the pond area was measured with a 50-meter tape. The rainfall and the air moisture were measured at Estação Meteorológica São Leopoldo. Rainfall corresponded to the total precipitation of the seven days prior to the survey.

2.3 Data analyses

The mean richness and abundance per collection was the average number of species and individuals observed in the five transects, respectively. Variations of the amphibians' mean richness and abundance over the studied period were quantified through analysis of variance (One-Way ANOVA). The variation of the amphibians richness and abundance between the hydrological phases was quantified through a *t*-test. Multiple regressions (General Linear Model – GLM) were used to determine all correlations between amphibians richness and abundance and the environmental variables (rainfall, air temperature, area, and water depth). The abundance values and the environmental variables were log transformed to remove heteroscedasticity.

Canonical Correspondence Analysis (CCA) was used to analyze the relationship between the composition and abundance of amphibian assemblage and the environmental variables (Ter Braak, 1986), using PC-ORD Version 4.2 (McCune and Mefford, 1999). The significance of the axes generated in the CCA was validated by the Monte Carlo test (using 5000 unrestricted iterations) (Ter Braak and Smilauer, 1998). The environmental variables utilized in the CCA were rainfall, air temperature, pond area and water depth, and they were centered and normalized. The biological variables were the amphibian species represented by five or more individuals found over the studied period. The variation of the amphibian composition between the two hydrological phases (with and without superficial water) was tested by Multi-Response Permutation Procedures (MRPP), considering the same species utilized in CCA.

3. Results

The pond presented two hydrological phases: a phase with surface water (between September-December 2004, and between May-August 2005, respectively), and a phase without surface water (between January-April 2005). The floodplain experienced three flash floods of different durations over the studied period: two floods were characterized as of short duration (two and five days), and one flood was characterized as of long duration (10 days) (Table 1). All three floods were enough to inundate all habitats of the floodplain system, besides the studied pond. The values of inundation area, mean water depth, and air and water temperature were presented in Table 1. A total of 17 anuran species distributed in six families (Bufonidae, Cycloramphidae, Hylidae, Leiuperidae, Leptodactylidae, and Microhylidae) was observed (Table 2). *Leptodactylus* gr. *ocellatus* (Linnaeus, 1758) (78.9%), *Physalaemus gracilis* (Boulenger, 1883) (52.6%), and *Physalaemus lisei* (Braun and Braun ,1977)(52.6%) presented the highest number of occurrence over the year. *Elachistocleis bicolor* (Valenciennes in Guérin-Menéville, 1838), *Leptodactylus fuscus* (Scheneider,1799), *Leptodactylus gracilis* (Duméril and Bibron, 1841), and *Physalaemus cuvieri* (Fitzinger, 1826) were observed in a single collection.

The Hylidae family represented 35.3% of the total species and 22.0 % of the individuals observed over the study period. (Table 2). *Rhinella fernandezae* (Gallardo, 1957) (Bufonidae) was the most abundant species, representing 24.7% of all collected individuals. The dominant species varied between the hydrological phases. While the leptodactylid *Leptodactylus* gr. *ocellatus* (36%) was the most abundant species in the first phase with surface water and the leiuperid *P. lisei* (37%) was the most abundant species in the phase without surface water, the hylid *Hypsiboas guentheri* (Boulenger, 1886) (34.9%) was the most abundant species in the second phase with surface water.

The amphibians richness and abundance did not change over the studied period ($F_{18,76} = 1.672$; P = 0.064, and $F_{18,76} = 1.151$; P = 0.323, respectively) (Figures 1, 2), and between the phases with and without surface water ($t_{17} = -1.085$; P = 0.293, and $t_{17} = -0.534$; P = 0.6, respectively). Environmental variables (air temperature, precipitation, water depth, and pond area) did not influence the amphibian richness (GLM, R² = 0.533; $F_{5,13} = 1.033$; P = 0.439) and abundance (GLM, R² = 0.429; $F_{5,13} = 0.586$; P = 0.711).



Figure 1. Anuran richness of an intermittent pond in Sinos River basin, between September (2004) and August (2005). Arrows = flood occurrence. Dashed lines = division of hydrological phases.

			_	Phase with surface water						
	Sept. 22	Sept. 24*	Sept. 27	Oct. 06	Oct. 20	Nov. 04	Nov. 10*	Nov. 22	Dec. 01	Dec. 16
Days after flood	-	0	3	12	26	41	0	12	21	36
Flood duration (days)	-	2	-	-	-	-	10	-	-	-
Air temperature (°C)	18	-	21.7	11	16.3	21.1	-	19.2	20.3	21.9
Water temperature (°C)	20	-	22.3	15	20.2	24.7	-	23.6	23.7	28.3
Pond area (m ²)	253.3	-	1,583.4	1,155.1	496	374	-	1,868.6	586.6	400.8
Air moisture (%)	83	-	91.9	77.3	80.4	81	-	78.4	81.3	75.9
Rainfall (mm)	155.1	-	143.1	19.8	64.0	20.2	-	8	0.5	1.1
. ,			Phase without surface water							
	Jan.	Jan. 26	Feb.	Mar.	Ma 2'	ar. 2	1	Apr. 06	Aj 2	or. O
Davs after flood	69	77	96	117	13	2		147	16	51
Flood duration (days)	-	-	-	-		-		_		-
Air temperature (°C)	19	15.6	20.1	25.2	2	0.2		24.1	2	21
Water temperature (°C)	-	-	-	-		-		-		-
Pond area (m ²)	-	-	-	-		-		-		-
Air moisture (%)	75.7	71.9	72.7	67.4	8	2.1		91	8	35.6
Rainfall (mm)	26.2	0	2.8	1.1	2	2.1		97.3	2	24.2
	Phase with surface water									
	Ma 22	May June 22* 01		lune 01	e June 15		,	July 26	Au 2	ıg. 2
Days after flood	C)	10		24			65	9	2
Flood duration (days)	5	5					-		-	
Air temperature (°C)	-		18.1		18.2		7.3		17.9	
Water temperature (°C)	-			17.9	1	8.9		11.3	1	9
Pond area (m ²)	-		1,2	34.3	59	5.9	:	54	1,93	2.7
Air moisture (%)	-			84	9	0.7	;	85.7	8	6.3
Rainfall (mm)	-			0	35.3			25	1	2.3

Table 1. Abiotic characteristics of an intermittent pond in Sinos River basin, from September 2004 to August 2005.

* = Flood occurrence.



Figure 2. Anuran abundance of an intermittent pond in Sinos River basin, between September (2004) and August (2005). Arrows = flood occurrence. Dashed lines = division of hydrological phases.

Table 2. Number of individuals (1 Eamil//Concise	total and	for sp	ecies) (Dhace	rans in	an inti rface w	ermitte	ant poi		sinos K	iver ba	Sin, fr(om Sej	ptemb6	21 2004	to Al	Bust 2	.con	Dhaca	with c	Infacto we	tor	
	1000	1000	1000					Nov			20				Apr Val	-		, of				A.12	LotoL
	sept. 23	Sept. 24*	sept. 27	0 CCI.	20 CCI.	N0V	10*.	22 VOV.	Lec.	uec. 16	Jan. 18	Jan. 26	гер. 14	Mar	Mar. , 22	, , , 0		viay 22*	1 v	June 15	July 26	22 Aug.	10131
BUFONIDAE																							
Rhinella femandezae	34	,	0	0	0	0		0	0	0	0	с	5	6	5	9	10		0	7	ę	0	82
CYCLORAMPHIDAE																							
Odontophrynus americanus	0		0	0	0	0		0	0	0	0	0	-	0	-	0	-		0	0	0	0	e
HYLIDAE																							
Dendropsophus sanborni	0		0	0	0	0	,	0	0	2	ი	2	0	0	0	2	0		0	0	0	0	6
Hypsiboas guentheri	0		2	0	0	0		0	0	0	0	0	0	0	0	0	0		16	œ	0	S	31
Hypsiboas pulchellus	0		0	0	0	0	·	0	-	0	0	0	0	0	0	0	0		2	0	0	0	с
Pseudis minuta	0		0	0	0	0	·	0	5	2	0	0	0	0	0	0	0		9	2	0	9	21
Scinax berthae	2	,	0	0	0	0	,	0	0	0	0	0	0	0	0	0	0	,	0	0	0	2	4
Scinax granulatus	0		4	0	0	0		-	0	0	0	0	0	0	0	0	0		0	0	0	0	5
LEIUPERIDAE																							
Physalaemus cuvieri	0		0	0	0	0	,	0	0	0	0	0	с	0	0	0	0	,	0	0	0	0	e
Physalaemus gracilis	-		-	0	0	0	,	5	0	0	4	5	2	1	-	. 	0	,	0	0	0	4	35
Physalaemus lisei	0		-	0	0	-		0	0	0	-	29	5	12	2	0	-		e	0	0	12	67
Pseudopaludicola falcipes	2		-	0	0	0		5	0	.	0	0	0	0	0	0	0		0	0	0	2	1
LEPTODACTYLIDAE																							
Leptodactylus fuscus	0	'	0	0	0	0	,	0	0	0	0	0	0	0	-	0	0		0	0	0	0	-
Leptodactylus gracilis	0	'	0	0	0	0		0	0		0	0	0	0	0	0	0	,	0	0	0	0	-
Leptodactylus latinasus	0		0	0	0	0		0	0	0	0	0	-	0	-	0	0		0	0	0	0	2
Leptodactylus gr. ocellatus	-	,	2	5	9	с	,	11	11	2	-	0	-	0	2	0	e	,	÷	-	0	с	53
MICROHYLIDAE																							
Elachistocleis bicolor	0		0	0	0	0	ï	0	0	0	0	0	-	0	0	0	0		0	0	0	0	~
Total richness	5	'	9	-	-	2	,	4	с	5	4	4	ø	e	7	e	4		5	4	-	7	17
Total abundance	40	'	7	5	9	4		22	17	œ	6	39	19	32	13	6	15		28	18	ო	34	332
* = Flood occurrences.																							

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The first two axes of the CCA explained, respectively, 23.9 and 13.1% of the total variation in the composition and abundance of the anuran assemblage (Table 3). The Monte-Carlo test showed that the collections sequence based on the species composition and the environmental variables was significant for the first two order axes (P < 0.05). Among the environmental parameters, pond area and water depth were negatively correlated with the first order axis (r = -0.7, and r = -0.9, respectively), and precipitation was positively correlated with the second axis (r = 0.763) (Figure 3). *H. guentheri, Pseudis minuta*

Table 3. Summary of Canonical Correspondence Analysis (CCA) results for the axes of the CCA ordination. Canonical coefficients and correlations coefficients of the environmental variables with the first two axes of the CCA.

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.563	0.309	0.109
Cumulative % variance of taxa	23.9	37.0	41.6
Pearson correlation:	0.967	0.836	0.622
species-environment data			
Intraset correlations			
Rainfall	0.326	0.763	-0.059
Pond area	-0.707	0.020	-0.358
Water depth	-0.897	0.180	-0.154
Air temperature	0.133	0.076	-0.271
Monte Carlo test (p)	< 0.001	0.013	0.165



Figure 3. Canonical Correspondence Analysis ordination biplot (CCA) with amphibian composition and abundance related to the studied environmental vectors.

(Günther, 1858), *L.* gr. *ocellatus* and *Pseudopaludicola falcipes*(Hensel, 1867) were more abundant in the period with higher pond area and water depth. *Dendropsophus sanborni* (Schmidt, 1944) was more abundant in the period with lower pond area and water depth. *Scinax granulatus* (Peters, 1871) and *R. fernandezae* were more abundant in the period with higher rainfall, and *P. lisei* and *P. gracilis* were more abundant in the period with lower rainfall (Figure 3).

The composition changed between phases with and without surface water (A = 0.082; P = 0.008). While the presence and abundance of the *Leptodactylus* gr. *ocellatus* (IV = 72.7; P = 0.035) characterized the phase with surface water, *Physalaemus gracilis* and *P. lisei* characterized the phase without surface water (IV = 67.6; P = 0.026, and IV = 71.5; P = 0.020, respectively).

4. Discussion

The anuran amphibian assemblages are strongly influenced by air temperature and amount of rainfall, mainly in the reproduction activity (Aichinger, 1987; Bernarde and Kokubum, 1999). In our study, rainfall and air temperature did not influence the richness and abundance of amphibian assemblage. These results could have been influenced by the regular distribution of the rainfall over the studied period and by no difference in air temperature between seasonal periods. This result does not underestimate the importance of the rainfall and air temperature for the amphibian assemblage, since the combinations of these parameters directly affect the presence and the absence of surface water in the studied pond. Besides, the environmental factors may different to influence each studied anuran species (see Moreira et al., 2007).

Intermittent ponds are characterized by extremes of flooding and total absence of surface water. Such hydrological extremes are regarded as disturbances in intermittent ponds in Southern Brazil (Maltchik, 2003), which can affect the biota in different ways. In this study, the anurans richness and abundance have not changed between hydrological phases. The maintenance of the richness and abundance of anurans along the study period was due to the change in species composition of the species between the studied phases. This result suggests that small wetlands resources were exploited by the anuran community along different hydrological phases. Seasonal variations in the occurrence and abundance of anurans have been noticed in wetlands due mainly to reproductive and physiological activities or to fluctuations from juvenile recruitment (e.g., Martori et al. 2005; Prado et al. 2005; Kopp and Eterovick, 2006).

Three species (*P. gracilis*, *P. lisei*, and *R. fernandezae*) helped to maintain the anurans richness and abundance over the phase without surface water. Due to terrestrial habits, *P. gracilis* and *P. lisei* were dominant species in the phase without surface water, and they were favored by substratum increase with litter along the pond area. According to Giaretta et al. (1997), the distribution of the litter frogs is connected with soil humidity, thus showing a tendency of frogs to be absent from drier places. *Rhinella fernandezae* was highly sedentary, frequently found in excavated shelters or cracks on the studied pond bottom. According to Gallardo (1969), such strategy ensures more safety for the individuals, since they leave their shelters only for feeding or reproduction. Despite the fact that the anuran community displayed strong resistance to periodical or seasonal drought, those results should be understood with some caution because the effects of a long-lasting drought could be different and, as such, better assessed.

The high number of observed individual of the foamnest species over the studied period in this survey follows the pattern observed in other Brazilian regions (Bernarde and Machado, 2001; Toledo et al. 2003). This prevalence may be related to some biological characteristics of this group of species. *Leptodactylus* gr. *ocellatus* and *P. lisei* showed fast larval development – about a month –, depending on the place (Eterovick and Sazima, 2004), and they laid their eggs on floating foam in the vegetation, thus preventing desiccation and making the transient pools' colonization easier. These species' reproductive strategies may have influenced their dominance in the studied habitat.

The high species richness and abundance of anurans observed in the intermittent pond (17 species and 332 individuals) showed the dependence on these habitats for their critical life-story functions. Small wetlands are particularly important for the amphibians (e.g., Semlitsch and Bodie, 1998), including the ones in Southern Brazil. Despite our results still preliminary (1 pond, and for a short period of time - 1 year), the high species richness observed pointing to the need to include small wetlands in the biodiversity conservation programs in southern Brazil, mainly when 90% of wetland systems have already been lost and the remaining ones are still at high risk due to the expansion of rice production and exotic Eucalyptus and pine plantations in southern Brazil (Maltchik, 2003). But these results should serve as hypotheses for long-term investigations in a larger area.

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