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CALLING PERIOD AND REPRODUCTIVE MODES IN AN ANURAN COMMUNITY OF A TEMPORARY POND IN SOUTHERN BRAZIL

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ABSTRACT: The objectives of this study were to describe the calling sites and identify the reproductive modes for the anuran assemblage in a temporary pond associated to a floodplain system in a Neotropical region in Southern Brazil. In this study we analyzed the influence of temperature, rainfall and hydroperiod on the calling activity of an anuran assemblage. A total of 19 amphibian collections were carried out over a year (from September 2004 to August 2005). Seventeen anuran species of six families were identified: Bufonidae (1), Cycloramphidae (1), Hylidae (6), Leiuperidae (4), Leptodactylidae (4) and Microhylidae (1); however, only fifteen species were observed in calling activity. Three reproductive modes were observed for the community, and the habitat use was temporally and spatially partitioned. 66.7% of the species had the generalized aquatic mode. The anurans presented a prolonged calling activity, and only *Chaunus fernandezae* presented an explosive pattern. While rainfall for the previous 24 h, air temperature and water depth influenced the calling activity of many anuran species over the studied period, daily rainfall, rainfall for the previous 24 h and water temperature did not influence it.

KEYWORDS: intermittent pond, anurans, reproductive pattern, flood plain system, southern Brazil.

INTRODUCTION

Anurans are especially dependent on water and/or atmospheric humidity for reproduction, because they are vulnerable to desiccation, at least in one phase of their lives (eg., egg, tadpole, or post metamorphic) (Prado *et al.*, 2005). This water dependence may lead to a high temporal and spatial competition for oviposition and calling sites (Crump, 1974). Some studies have documented that anurans can differ in habitats use for reproduction, calling sites and/or reproductive activity period (Rossa-Feres and Jim, 1994; Prado *et al.*, 2005; Vasconcelos and Rossa-Feres, 2005; Kopp and Eterovick, 2006).

Temporal segregation in the use of breeding ponds can be an important reproductive isolation mechanism for species that breed synchronically (Bertoluci and Rodrigues, 2002). Calling sites, features of advertisement call, and daily period of calling activities are also isolation mechanisms (Pombal Jr., 1997). Vegetation cover, flow regime and wetland hydroperiod influence the species segregation in frog assemblages (Heyer, *et al.*, 1990; Arzabe *et al.*, 1998).

The anuran assemblages are strongly influenced by air temperature, total amount of rainfall and hydroperiod (Duellman and Trueb, 1994; Pechmann *et al.*, 1989; Rodrigues *et al.*, 2003). Temperature influences several physiological processes of amphib-

ians, such as water balance, calling, metamorphosis, development and immune response (Rome *et al.*, 1992). Rainfall is the main environmental cue that initiates reproduction for most pond-breeding anurans (Eterovick and Sazima, 2000; Prado *et al.*, 2005; Kopp and Eterovick, 2006). While anurans tend to reproduce throughout the year in sites with constantly high temperature and humidity, in seasonal regions reproduction is associated with the rainy and hot phases (Duellman and Trueb, 1994). The hydroperiod is one of the most important factors determining species richness, productivity and habitat suitability for pond-breeding amphibians (Babbitt and Tanner, 2000). Variations in the water level may affect the abundance and diversity of species of amphibians in the wetland system (Pechmann *et al.*, 1989). Interspecific difference in life history traits among anurans, such as length of larval developmental period and ability to breed successfully with predators, limits the range of wetlands within each species which can breed successfully (Babbitt and Tanner, 2000). Species with long larval periods are excluded from sites with short hydroperiods. The calling activities of many anuran species of temporary ponds are associated with water availability (Eterovick and Sazima, 2000).

Anuran has one of the greatest diversity of reproductive modes among the vertebrates (Duellman and Trueb, 1994). However, studies performed on

the reproductive patterns and habitat use in anurans assemblage in open environments are scarce (Arzabe, 1999; Eterovick and Sazima, 2000; Prado *et al.*, 2005; Vasconcelos and Rossa-Feres, 2005). Such gap becomes absolutely worrying in Brazil, a country with the greatest amphibian diversity in the world (IUCN, 2004).

The objectives of this study were to describe the calling sites and identify the reproductive modes for the anuran assemblage in a temporary pond associated to a floodplain system in a Neotropical region in Southern Brazil. Here we analyzed the influence of temperature, rainfall and hydroperiod on the calling activity of an anuran assemblage. Our approach is an exploratory analysis to describe the different calling sites and the importance of some environmental parameters on calling activities of an anuran community in a temporary pond in southern Brazil, for further hypothesis testing.

MATERIAL AND METHODS

Study Area

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

The studied site is a temporary pond (29°42'16"S, 50°59'21"W, 21 m elevation), fed by water from rainfall, runoff and flood events from the Sinos River. It 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 riparian predominated: 60% of native woodland (mainly *Mimosa bimumcronata*) and 40% of grasslands.

Field activities

A total of 19 amphibian collections were carried out over a year (from September 2004 to August 2005). The survey was made in five transects distributed at random within the studied lake (30 x 2 m). The sampling efforts for each transect lasted twenty minutes, totalizing 1h40min of sampling per collection. All collections were made from 6:00 pm to 12:00 pm. The individuals were located visually or acoustically over the transects (Crump and Scott, 1994). The distance from surface water edge, height above the ground and type substrate were measured for each calling male observed. The reproductive period of each species was determined on calling males and presence of amplexant pairs. The species were grouped into their respective reproductive modes according *sensu* Haddad and Prado (2005).

Air and water temperature were measured just after dusk with 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. Rainfall was measured at the Estação Meteorológica de São Leopoldo, RS.

Statistical analyses

To test for a possible association between calling activity and abiotic factors, a stepwise multiple regression with backward selection was carried out. We compared the number of calling species of each reproductive mode with the daily rainfall (from 00h00 to 24h00), total rainfall for the previous 24-hour period, total rainfall for the previous 72-hour period, air temperature, water temperature and water depth.

RESULTS

The pond presented two hydrological phases over the studied period: a phase with surface water (between September-December 2004, and between May and Aug 2005, respectively), and a phase without surface water (between January and April 2005) (Figure 1). The floodplain experienced three floods (September 24, May 22, November 10).

Seventeen anuran species belonging to six families (Bufonidae, Cycloramphidae, Hylidae, Leiuperidae, Leptodactylidae and Microhylidae) were identified, however only fifteen were observed in activity at the study site. We registered three reproductive modes, *sensu* Haddad and Prado (2005), as follows (Table 1):

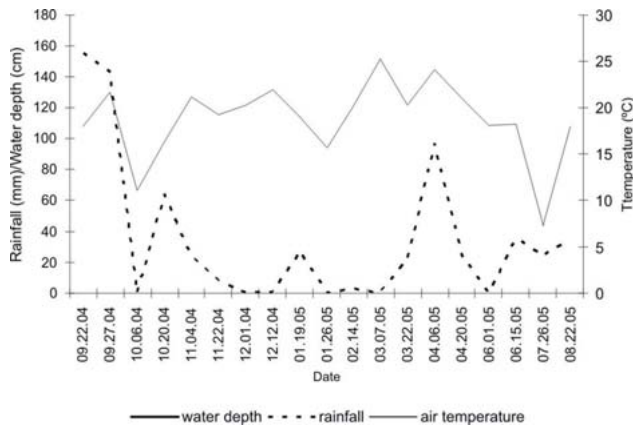


FIGURE 1. Total rainfall (mm) of the seven days previous to sampling, medium water depth (cm) and air temperature (°C) at sampling nights.

Mode 1, includes species with eggs and exotrophic tadpoles in lentic water (10 species; 4 families); mode 11 includes species that lay eggs in foam nests on the water surface (4 species; 2 families); and species building foam nests with eggs and early larval stages in subterranean constructed chambers; subsequent to flooding, exotrophic tadpoles in ponds, comprised the mode 30 (1 species).

No species of mode 1 called during the months of July and October, when the pond contained wa-

ter (Table 2). Only one male of *Hypsiboas guentheri* called when the pond was dry, from shrub branches on a rainy night (06 April 2005). *Chaunus fernandezae*, *Dendropsophus sanborni* and *H. pulchellus* called at one night only, at misty or rainy nights. The other species called in two or more nights of the study period. Calling activity peaks were recorded in September 2004 and August 2005, when seven and eight species called, respectively.

In mode 11, *Physalaemus gracilis* was the specie that called for a more extensive period (Table 2). *Leptodactylus ocellatus* was the single specie that did not call in September. No species were found in calling activity when the pond was dry.

L. gracilis was the single species of mode 30 that was in calling activity, although some froglets of *L. fuscus* and *L. latinasus* had been observed visually.

The anuran species called in different periods of the year, and the habitat use was temporally and spatially partitioned by the studied anuran community. The highest calling activities were observed in September (2004), November (2004) and August (2005) (Table 2). A total of 11 species were observed in calling activity in September. At least five species called floating close to the bank, at shallow sites, and among aquatic vegetation (*C. fernandezae*, *Elachistocleis bicolor*, *P. cuvieri*, *P. gracilis* and *P. lisei*). Other species,

TABLE 1. Anuran species, calling sites, and reproductive modes (*sensu* Haddad and Prado, 2005) registered in a temporary pond in the Sinos river floodplain, Southern Brazil. 1 = Aquatic oviposition, 11 = Oviposition in aquatic foam nest and aquatic tadpoles, 30 = Oviposition in terrestrial foam nest inside subterranean chambers, and aquatic tadpoles.

Families/Species	Reproductive mode	Calling site
Bufonidae		
<i>Chaunus fernandezae</i>	1	shallow water or floating vegetation
Cycloramphidae		
<i>Odontophrynus americanus</i>	1	shallow water or wet ground
Hylidae		
<i>Dendropsophus sanborni</i>	1	shrub branches up to 1.5 m height above water
<i>Hypsiboas guentheri</i>	1	shrub branches up to 1.5 m height above water
<i>Hypsiboas pulchellus</i>	1	shrub branches up to 1.0 m height above water
<i>Pseudis minuta</i>	1	floating among the aquatic vegetation
<i>Scinax berthae</i>	1	shrub branches up to 0.5 m height above water
<i>Scinax granulatus</i>	1	shrub branches up to 1m height
Leiuperidae		
<i>Physalaemus cuvieri</i>	11	floating among the aquatic vegetation
<i>Physalaemus gracilis</i>	11	floating among the aquatic vegetation
<i>Physalaemus lisei</i>	11	floating among the aquatic vegetation or open water
<i>Pseudopaludicola falcipes</i>	1	wet ground close to vegetation
Leptodactylidae		
<i>Leptodactylus ocellatus</i>	11	open water
<i>Leptodactylus gracilis</i>	30	ground among grass clumps
Microhylidae		
<i>Elachistocleis bicolor</i>	1	shallow water

e.g., *H. guentheri*, *Scinax berthae* and *S. granulatus*, called on shrub branches above water. *L. gracilis* called from the ground, among grass clumps in open areas at the edge of the pond. Males of *Odontophrynus americanus* and *Pseudopaludicola falcipes* called on waterlogged ground close to grass tufts.

A total of ten species were observed in calling activity in November. While *Pseudis minuta* called on the emergent aquatic vegetation with leaves on the water surface, *L. ocellatus* called in the middle of the pond, generally in places where the aquatic vegetation was absent. The hylids, *H. guentheri* and *H. pulchellus* used the same calling sites in August 2005 – both species called in shrub branches, 100 to 150 cm above water.

As calling activity was not registered in the drought period of the pond (except for only a sin-

gle male of *H. guentheri*), we used only data from months when the pond had held water in the analyses. The backward stepwise multiple regression models revealed that the measured variables of rainfall for the previous 24 h, air temperature and water depth contributed best to explain calling activity (Table 3), although in different ways for each species. Rainfall for the previous 24 h was found to contribute significantly for the variation in the calling activity in modes 1 and 11. Air temperature was significant in the regression model of mode 30. Water depth was negatively correlated ($\beta = -0.025$; $p = 0.001$) with calling activity in mode 30 and positively in mode 1 ($\beta = 0.082$; $p = 0.002$). Rainfall for the previous 72 h, daily rainfall and water temperature had no apparent effect on calling activity of any mode.

TABLE 2. Calling period of anuran species in a temporary pond in the Sinós river floodplain, Southern Brazil (+) = calling activity (*) = presence of amplexant pairs.

Species	Date																		
	22 Sep	27 Sep	06 Oct	20 Oct	04 Nov	22 Nov	01 Dec	16 Dec	19 Jan	20 Jan	14 Feb	07 Mar	22 Mar	06 Apr	20 Apr	01 Jun	15 Jun	26 Jul	22 Aug
Mode 1																			
<i>C. fernandezae</i>	+	*																	
<i>D. sanborni</i>								+											
<i>H. guentheri</i>	+	+												+		+	+		+
<i>H. pulchellus</i>																			+
<i>P. minuta</i>						+	+									+	+		+
<i>S. berthae</i>	+	*	+		+	+										+	+		+
<i>S. granulatus</i>	+	+			+	+													+
<i>E. bicolor</i>	+	*	+			+	+												+
<i>O. americanus</i>	+	*	+																+
<i>P. falcipes</i>	+	*	+		+	+		+											+
Mode 11																			
<i>L. ocellatus</i>						+	+												
<i>P. cuvieri</i>	+				+														
<i>P. gracilis</i>	+	+		+		+										+			+
<i>P. lisei</i>	+				+											*			+
Mode 30																			
<i>L. gracilis</i>	+			+	+			+											
Total of species	11	7	0	2	6	7	3	3	0	0	0	0	0	1	0	5	3	0	10

TABLE 3. Backward stepwise multiple regression results comparing the number calling species in each reproductive mode, from a temporary pond in Sinós river floodplain, with daily rainfall (rain), total rainfall for the previous 24h period, total rainfall for the previous 72 h period, air temperature, water temperature, and water depth. Only values of included variables are shown.

	Variable	β	R ²	df	F	P
Richness of mode 01	rain 24 h	0.144	0.854	2	26.365	< 0.001
	water depth	0.082				0.002
Richness of mode 11	rain 24 h	0.043	0.533	1	11.395	0.007
Richness of mode 30	air temperature	0.088	0.734	2	12.387	0.004
	water depth	-0.025				0.001

DISCUSSION

Many factors have been suggested to influence patterns of anurans distribution at specific sites, such as predation (Eterovick and Sazima, 2000), hydrological cycles (Arzabe *et al.*, 1998), habitat duration (Eterovick and Fernandes, 2002), and rainfall (Arzabe, 1999; Toledo *et al.*, 2003). Toft (1985) noticed that the resource partitioning in anurans results from many different causes, each one acting at a different intensity.

In tropical regions rainfall seems to be the main factor controlling anuran reproductive patterns (Heyer, 1973) because frogs are vulnerable to desiccation and extremely dependent on water availability. In our study, air temperature and water availability influenced the calling activity of the anuran assemblage, although similarities and differences among the 15 species have been observed. The daily rainfall did not influence the calling activity of anurans; however, rainfall for the previous 24 h was positively correlated with calling activity of species of modes 1 and 11. Several studies have demonstrated that most anurans species had started calling at the onset of the rains (Eterovick and Sazima, 2000; Prado *et al.*, 2005; Kopp and Eterovick, 2006).

Rainfall for the previous 24 h and water depth influenced the calling activity of mode-1 species. Correlation with water depth reflects the importance of the availability of water for species of mode 1, since these frog species do not have mechanisms to avoid egg's dehydration. Many species of mode 1 were observed in calling activity in the water, floating among the aquatic macrophytes (*C. fernandezae*, *P. minuta* and *E. bicolor*) or on branches over the water (*D. sanborni*, *H. guentheri* and *S. berthae*).

The calling activity of *L. ocellatus*, *P. cuvieri*, *P. gracilis*, and *P. lisei* (mode 11) were correlated with the rainfall for the previous 24 h. These species are not so dependent of ponds for reproductive activities because eggs and larvae are protected within the foam nests (Heyer, 1969). However, the presence of surface water is important, since they used it as calling sites, deposition place of foam nests or tadpoles' development.

The calling activity of *L. gracilis* (mode 30) was negatively correlated with water depth and positively correlated with air temperature. This species and others of the *fuscus* group do not need water for egg laying and also count on the protection of the foam nest against dehydration (Heyer, 1969). However, the humidity provided by the rainfall is highlighted as an

important factor for terrestrial reproductive modes (Pombal Jr. and Haddad, 2005) because adults and foam nests can not withstand long periods of drought. Species in the *fuscus* group usually call on the ground in open grasslands subject to floods (Achaval and Olmos, 1997; Eterovick and Sazima, 2004; Kwet and Di Bernardo, 1999). In our study, *L. gracilis* called on the ground among grasses, in an island formed in the middle of the pond due to water level decrease. The increase on the water level could be hiding the calling sites of this species, so inhibiting the calling activity. Three species of the *fuscus* group (*L. fuscus*, *L. gracilis* and *L. latinasus*) were observed calling for a larger period of time (A. Lace, personal observation) in an open grassland field near the studied pond.

Anuran species more resistant to desiccation, with more generalized reproductive modes (e.g., mode 1) or modes adapted to insolation (e.g., modes 11 and 30) are more favored in seasonal environments. In our study, the mode 1 was more frequent (66.7%). This result is in accordance with some researches developed in other seasonal environments in South America. Perotti (1997) found 50% of the species, with generalized reproductive mode, in the Chaco region. Eterovick and Sazima (2000) and Prado *et al.* (2005) found 53.8% and 62.5% of the species, respectively, with reproductive mode generalized to other Brazilian regions.

The proportion of species with eggs in foam observed in our study was similar to those reported in Caatinga and Cerrado (Arzabe, 1999; Eterovick and Sazima, 2000). Although frogs exhibit a great diversity of reproductive modes, only three modes were observed in our study. Many authors have suggested that diversity is associated to environmental factors, such as high atmospheric moisture, high temperature, and absence of seasonality (Duellman, 1989; Hödl, 1990).

Several anuran species presented prolonged calling activity patterns, coinciding with the presence of surface water. Only *C. fernandezae* presented an explosive pattern. Wells (1977) stated that explosive breeders call for few days and show associated behaviors (e.g., amplexant pairs, overlap of calls, active search for females). Besides *C. fernandezae*, other species called for few days at the study site, but calling males did not aggregate in large numbers (*D. sanborni*, *H. pulchellus* and *L. ocellatus*). Many amplexant pairs and overlap of calls were observed in two species (*C. fernandezae* and *H. guentheri*). However, *H. guentheri* called during two months or more, exhibiting an intermediate pattern of reproduc-

tion (between explosive and prolonged). The recorded calling activity periods of the anurans species studied coincided with periods recorded for a given species in others areas, such as *L. ocellatus*, *P. cuvieri* and *P. gracilis*, at São José dos Pinhais (Conte and Rossa-Feres, 2006), and in the state of São Paulo, such as *D. sanborni*, *P. falcipes* and *E. bicolor* (Bernarde and Kokubum, 1999).

Differences on partitioning of calling sites are important reproductive isolation mechanisms (Cardoso and Haddad, 1992; Pombal Jr, 1997). Many species, mainly of the families Bufonidae, Leiuperidae and Microhylidae showed overlap in the calling site use over the studied period. They were observed in calling activities floating close to the bank, at shallow sites. Most species of hylids used calling sites at different heights: in surface water (*P. minuta*) and up to 2 m high (*D. sanborni*, *H. guentheri*, *H. pulchellus*, *S. berthae* and *S. granulatus*). Adhesive discs adapt these frogs to climb different types of substrates at varied heights (Pombal Jr., 1997). On the other hand, bufonids, leiuperids and microhylids of this study would be subject to calling site overlap, due to the absence of adhesive discs and climb ability.

The calling activity does not mean necessarily a breeding event (Scott Jr. and Woodward 1994). Information about amplexant pairs, eggs, and tadpoles are useful tools to better determine the reproductive period. We could not confirm the breeding activity of five species (*D. sanborni*, *H. pulchellus*, *S. granulatus*, *P. cuvieri* and *L. gracilis*) of the 15 species observed in calling activities. These species were rare at the study site; however, some were abundant and presented different calling sites (e.g., *D. sanborni*, *H. pulchellus*) in a permanent pond in an open area of the same floodplain. Pombal Jr. and Haddad (2005) suggested that the same species can exhibit different temporal patterns or behavioral strategies (that may be associated with density) in different environments.

In spite of our study to be of short duration, it shows that in tropical seasonal environments anuran life history events are associated with fluctuations in rainfall and very dependent on the weather conditions, such as floods, as previously reported by other studies (e.g., Bertoluci and Rodrigues, 2002; Gottsberger and Gruber, 2004; Prado *et al.* 2005)

RESUMO

Os objetivos deste estudo foram descrever os sítios de vocalização e identificar os modos reprodutivos de uma comunidade de anuros em uma lagoa

temporária associada a uma planície de inundação na região neotropical do sul do Brasil. Neste estudo nós analisamos a influência da temperatura, precipitação e hidroperíodo sobre a atividade de vocalização da comunidade de anuros. Um total de 19 coletas foi realizado durante um ano (setembro de 2004 a agosto de 2005). Dezesete espécies de anuros de seis famílias foram identificadas: Bufonidae (1), Cycloramphidae (1), Hylidae (6), Leiuperidae (4), Leptodactylidae (4) e Microhylidae (1), mas somente 15 espécies foram observadas em atividade de vocalização. Três modos reprodutivos foram observados para a comunidade e uso do habitat foi temporalmente e espacialmente partilhado. 66,7% das espécies tinham o modo aquático generalizado. Os anuros apresentaram uma atividade de vocalização prolongada e somente *Chaunus fernandezae* apresentou um modelo explosivo. Enquanto que a precipitação nas 24 h prévias, temperatura do ar e profundidade da água influenciaram a atividade de vocalização de muitas espécies no período de estudo, precipitação diária, precipitação nas 72 h prévias e temperatura da água não influenciaram.

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