

## Breeding biology of *Physalaemus centralis* Bokermann, 1962 (Anura: Leptodactylidae) in southeastern Brazil

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(Accepted 26 May 2006)

### Abstract

*Physalaemus centralis* is widely distributed in areas of cerrado vegetation in South America. Here we describe the temporal variation in activity and explore eventual effects of climatic conditions on activity, calling sites, territorial behaviour; courtship behaviour, egg clutches and tadpole development of *P. centralis* in southeastern Brazil. Field observations were made from January 1996 to February 1997. Vocalisations and reproductive activity were restricted to the rainy season, with a peak of activity in October. The number of calling males was significantly correlated with relative humidity, mean air temperature, and air pressure values of the previous day. Courtship is simple and did not include any physical contact before amplexus; furthermore, females seem to choose their mates. Amplexant pairs were observed from mid-October to mid-December. Most foam nests observed were anchored within the vegetation. The number of eggs in the foam nests ranged from 1549 to 2405. Tadpoles were mainly found in shallow parts of the water from October to mid-January. The reproductive biology of *P. centralis* is similar to that of closely related species of the *P. cuvieri* group, suggesting conservatism in this lineage.

**Keywords:** Anura, Brazil, Leptodactylidae, *Physalaemus centralis*, reproduction

### Introduction

The genus *Physalaemus* currently includes 46 species that occur from southern Mexico to northern Argentina (Frost 2005; Ron et al. 2005). Information on the reproductive biology of *Physalaemus* species is available for nine taxa: *Physalaemus atlanticus* (Haddad and Sazima 2004); *P. cuvieri* (Bokermann 1962; Cardoso 1981; Barreto and Andrade, 1995), *P. enesefae* (Tarano and Ryan 2002; Tarano and Herrera 2003); *P. fuscomaculatus* (Giarretta and Menin 2004); *P. ephiphifer* (Hödl 1990), *P. signifer* (Weber and Carvalho e Silva 2001; Wogel et al. 2002), *P. spiniger* (Haddad and Pombal, 1998), *P. rupestris* (Nascimento and Carvalho 2001), and *P. pustulosus* (e.g. Ryan 1985). All members in the genus exhibit

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Published 28 August 2006

ISSN 0022-2933 print/ISSN 1464-5262 online © 2006 Taylor & Francis  
DOI: 10.1080/00222930600835193

axillary amplexus, lay numerous eggs in foam nests, and have pond-type larvae (e.g. Lynch 1971).

Patterns of reproduction in the genus *Physalaemus* vary from extremely explosive, such as in *P. nattereri* and *P. fuscumaculatus* (Giaretta and Menin 2004; Brasileiro et al. 2005), to prolonged, as in *P. cuvieri* (Cardoso 1981; Barreto and Andrade 1995). *Physalaemus centralis* is widely distributed in cerrado (savanna-like) areas of South America, ranging from southeastern and central Brazil to northeastern Paraguay (Bokermann 1965; Frost 2005). It is a common species with a conspicuous vocalization and breeds in several habitats in the vicinity of human settlements. However, the reproductive biology of *P. centralis* has not been studied in detail. Sparse information is available, such as the descriptions of adults (Bokermann 1965) and tadpoles (Rossa-Feres and Jim 1993); and male combat has also been described (Brasileiro 1998).

Here we report on the reproductive biology of a population of *P. centralis* (Figure 1) in southeastern Brazil. Our main objectives were: (1) to describe the activity pattern and to verify the possible influences of climatic conditions on reproductive activity; (2) to describe calling sites and territoriality in this species; and (3) to describe courtship behaviour, egg clutches, and development of tadpoles.

### Material and methods

Fieldwork was carried out at the CEPTA/IBAMA (Centro de Pesquisa e Treinamento em Aquicultura) fish hatchery in the municipality of Pirassununga (47°25'28" W, 21°59'52" S),

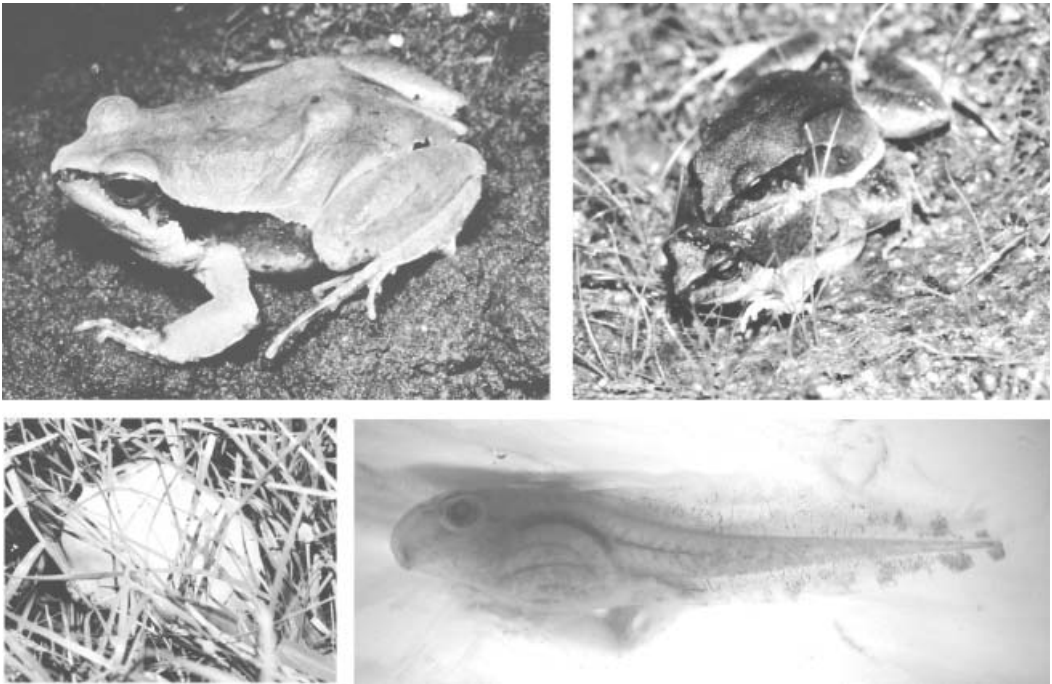


Figure 1. Aspects of the life cycle of *Physalaemus centralis*: adult male (upper left); axillary amplexus (upper right); foam nest (bottom left); tadpole (bottom right).

São Paulo State, southeastern Brazil. The original vegetation at this site was “cerrado” (a savanna-type vegetation; see Ratter et al. 1997). However, it has been highly modified for agriculture. The climate is subtropical; mean annual rainfall is 1280 mm, with a dry winter from April to September (5–166 mm per month), and a wet summer from October to March (122–285 mm). In 1996, minimum monthly temperature varied from 9 to 16°C in the dry season and from 17 to 21°C in the wet season; the maximum temperature varied from 25 to 30°C in the dry season and from 28 to 32°C during the wet season. Occasional frosts usually occur in June and July.

Our observations were made in a large rectangular fish hatchery tank (60 × 22 m). Water volume varied during the study period, according to precipitation. In the peak of the rainy season, one end of the tank was *ca* 10 cm and the other one *ca* 50 cm deep. Vegetation inside the tank consisted mainly of Poaceae, Cyperaceae, Juncaceae, and Pontederiaceae. Additional observations were made in five similar tanks at the same site. Two other species of *Physalaemus* (*P. cuvieri* Fitzinger, 1826 and *P. nattereri* Steindachner, 1863) and 11 additional species of frogs (*Bufo schneideri* Werner, 1894; *Dendropsophus minutus* Peters, 1872; *D. rhea* Napoli and Caramaschi, 1999; *D. sanborni* Schmidt, 1844; *Hypsiboas albopunctatus* Spix, 1824; *Scinax fuscomarginatus* (Lutz, 1925); *S. fuscovarius* (Lutz, 1925); *S. similis* (Cochran, 1952); *Leptodactylus furnarius* Sazima and Bokermann, 1978; *L. fuscus* (Schneider, 1799); *L. jolyi* Sazima and Bokermann, 1978; *L. podicipinus* (Cope, 1862); *Pseudopalodicola cf. falcipes* (Hensel, 1867)), bred in the same ponds as *P. centralis*.

From January 1996 to February 1997, the breeding sites were visited three to four nights (17:00 to 02:00 h) each week during the rainy season and every other week during the dry season (total=58 nights). For 1 h each night the tank was thoroughly inspected and the number of males and females, their location, behaviour, and number of pairs in amplexus were recorded. The all-occurrences method was used to describe adult behaviour (Lehner 1979). Water and air temperatures were measured hourly. We measured the snout-vent lengths (SVL) of 36 individuals (27 males, nine females) with a calliper. Five amplexant pairs were collected and housed in aquaria with water and vegetation until spawning. Part of each clutch was preserved in 70% alcohol, while the remaining eggs were reared to metamorphosis. Tadpoles were fed with lettuce once a week.

Foam nests were measured (height, length, and width) with a metric tape (accuracy 1 mm), and the presence/absence of larvae was recorded. Eight entire clutches were fixed. The eggs were counted and the diameters of 10 eggs from each nest were measured under a microscope.

Tadpoles were sampled weekly from the tank and fixed in 5% formalin. Gosner's (1960) table was used to identify larval stages of the total of 186 tadpoles sampled. Larval stages were divided into three classes (Barreto and Andrade 1995): (1) initial phase, from hatching to stage 25; (2) intermediate phase, between stages 26 and 39; and (3) final phase, between stages 40 and 46. Total length of each tadpole was measured with a calliper (accuracy 0.05 mm).

To identify correctly tadpoles of *P. centralis* we used a hand-held magnifying lens because two closely related species (*P. centralis* and *P. cuvieri*) occur at the same pond. The tadpoles of these species differ in details of the mouth structures, including tooth row arrangement (2(2)/2 in *P. centralis* and (2)/3(1) in *P. cuvieri*) and lower jaw shape (V-shaped in *P. centralis*; U-shaped in *P. cuvieri*; Rossa-Feres and Jim 1993; Rossa-Feres and Nomura 2006).

Daily meteorological data were obtained at Academia da Força Aérea, located 5 km from the study site. Voucher specimens (adults and tadpoles) were deposited in the Museu de História Natural, Universidade Estadual de Campinas, São Paulo, Brazil.

For statistical analyses we used simple linear regression, Mann–Whitney, and Pearson correlation with  $\alpha=0.05$  (Zar 1996).

## Results

### *Adults and reproductivity activity*

Mean SVL of *Physalaemus centralis* males was 36.30 mm (SD=2.40 mm, range 32.7–39.2 mm,  $n=27$ ) and of females 34.5 mm (SD=2.7 mm, range 32.7–38.1 mm,  $n=9$ ). Males and females did not differ in body size ( $U=77$ ;  $P=0.27$ ). Mean SVL of amplexant males (mean=36.7 mm, SD=1.9 mm, range 32.8–38.8 mm,  $n=8$ ) was not significantly different from that of non-amplexant males (mean=35.21 mm, SD=2.48 mm, range 33.4–38.9 mm,  $n=19$ ;  $U=75.5$ ;  $df=25$ ;  $P=0.24$ ).

The reproductive season of *P. centralis* began immediately after the first rainfalls in early September 1996 and finished in late December of the same year (Figure 2). No reproductive activity was observed during the remaining months (January to August 1996 and January and February 1997). The number of calling males was highly variable throughout the breeding season with a peak in October (Figure 2). In the days with no rainfall, the number of calling males decreased, and increased again 1 or 2 days later. The number of calling males was significantly correlated with relative humidity, mean air temperature of the day of observation, and air pressure values of the previous day, but not with those of the same day. Calling activity was also independent of rainfall on the same day or the previous day (Table I). Amplexant pairs were observed from mid-October to mid-December.

Calling activity started at dusk, usually between 17:00 and 22:00 h. The time of the first calls emitted each night was positively correlated with sunset ( $r=0.862$ ;  $P<0.01$ ;  $n=19$ )

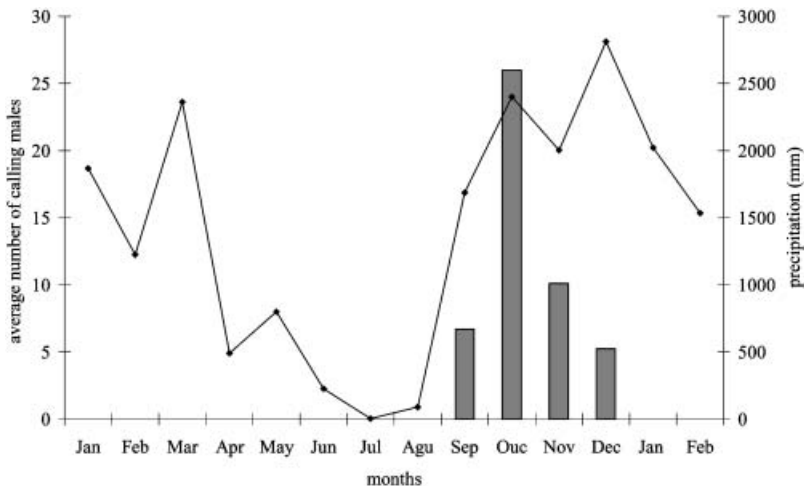


Figure 2. Monthly average number of calling males of *Physalaemus centralis* from January 1996 to February 1997 (bars) and total monthly precipitation (lines) at the study site, municipality of Pirassununga, São Paulo State, Brazil.

Table I. Relationships between abiotic factors and maximum number of calling males of *Physalaemus centralis*.

Abiotic factors	<i>n</i>	<i>r</i>	<i>P</i>
Relative humidity <sup>a</sup>	64	0.244	<0.05
Relative humidity <sup>b</sup>	35	-0.113	>0.05
Air pressure <sup>a</sup>	48	0.240	>0.05
Air pressure <sup>b</sup>	48	0.391	<0.05
Rainfall <sup>a</sup>	64	0.120	>0.05
Rainfall <sup>b</sup>	64	0.219	>0.05
Average temperature	64	0.241	<0.05

<sup>a</sup>Data measured on the observation day; <sup>b</sup>data measured on the day before. Data were measured at 20:00 h.

and air temperature at 22:00 h ( $r=0.707$ ;  $P<0.05$ ;  $n=18$ ). After dusk, the number of calling males gradually increased and reached its peak at 20:00–21:00 h, when calling activity began to decrease until approximately 02:00 h (Figure 3). The duration of daily calling activity varied throughout the breeding season and was correlated with the maximum number of calling males observed each night ( $r=0.55$ ;  $P<0.05$ ;  $n=20$ ) and with air temperature ( $r=0.99$ ;  $P>0.01$ ;  $n=20$ ) and water temperature ( $r=0.94$ ;  $P>0.01$ ;  $n=20$ ) at 20:00 h.

#### Calling site and territoriality

Male *P. centralis* called on the ground, usually near the shore or on exposed sections of the tank bottom. No male was observed changing its calling site during the calling period on the same night; however, individual location changed as water level changed on different days; when water depth increased, males moved towards the shore.

Males defended puddles used as calling sites. The territorial behaviour of resident males was escalated, and varied in relation to intruder behaviour. When an intruder called from a distance under about 1.5 m, the resident emitted territorial calls. Two behavioural responses were observed in this case: either (1) the intruder also emitted territorial calls and

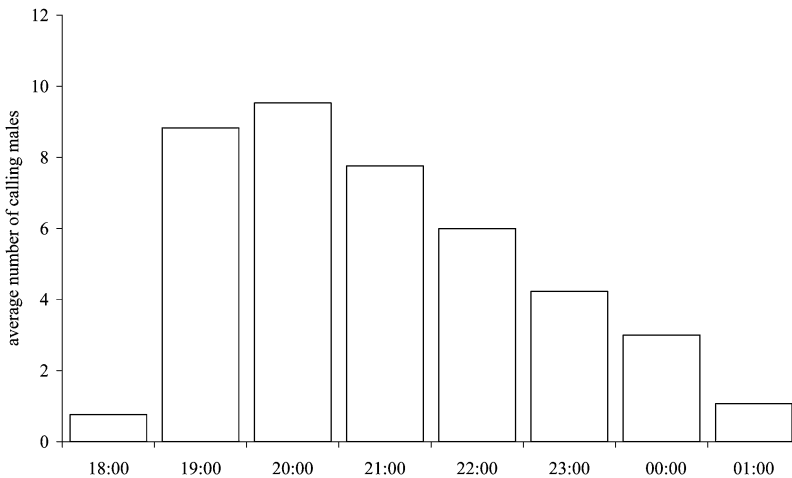


Figure 3. Average number of calling males during the nights at the study site, municipality of Pirassununga, São Paulo State, Brazil.

the interaction could last for up to 4 h; or (2) the intruder moved away or stopped calling. When intruders got very close to residents (0.3 m or less), they emitted another type of territorial call. In this case the intruder either would move away or stop calling. No intraspecific fights were observed, although the dense vegetation on the bottom of the hatchery could have impeded the observation of possible fights. In empty tanks with small rainy puddles but no plants, fights were observed on rainy nights with high densities of calling males, but these fights were not preceded by encounter calls (Brasileiro 1998).

#### *Courtship and oviposition behaviour*

Females ( $n=4$ ) were observed moving through the tank on four occasions. They slowly approached males and stopped moving at short distances (5–50 cm) from them. These movements lasted from 30 min to over 1 h. Males remained at their calling sites and apparently did not notice the approaching females. On two occasions, females moved away from males after staying for approximately 30 min at a distance of 5 cm from them. No males were observed intercepting moving females. On two other occasions, females approached calling males to a distance  $<3$  cm. A few seconds later, in both cases, the male stopped calling and clasped the female in axillary amplexus, without any previous contact. Pairs in amplexus ( $n=8$ ; Figure 1) were observed between 19:00 and 22:00 h, most of them after 21:00 h ( $n=6$ ).

After being amplexed by a male, females either remained at the site ( $n=2$ ) or carried the male to a site nearby ( $n=2$ ). Oviposition occurred only when calling activity finished or significantly decreased, usually around 02:00 h. Oviposition lasted on average 28 min ( $n=4$ ; 21–33 min), with periods of activity and resting. Each sequence of oviposition behaviour started with the female assuming an arched back posture (see Hödl 1992). Immediately after the female assumed this posture, the male drew its legs forward and pushed its feet, resulting in a pronounced hunched position, such that the cloacae of male and female were juxtaposed, the male beat its legs alternately, stirring the water and air with the eggs, jelly, and sperm to produce the foam nest. The pair remained motionless until the female restarted the sequence and repeated the cycle. During foam production, females did not move. No special signal to terminate amplexus was observed. Subsequent to oviposition, the pair separated and individuals moved away in different directions.

#### *Egg clutches and tadpoles*

Foam nests observed were anchored within the vegetation ( $n=15$ ; Figure 1) or in open water ( $n=5$ ). These were elliptically shaped, with mean length 10.0 cm (SD=2.2 cm; range 5.5–13.0 cm), mean width 7.4 cm (SD=1.6 cm, range 5.5–9.5 cm), and mean height 1.8 cm (SD=0.50 cm, range 1.0–3.0 cm,  $n=20$ ). Nests were white, formed by small air bubbles and were of hard consistency; the foam did not dissolve completely in 5% formalin. Mean number of eggs was 1842 (SD=354, range 1549–2405,  $n=8$ ). Eggs were white with mean diameter 1.3 mm (SD=0.13 mm, range 1.0–1.5,  $n=80$ ). Just after oviposition, eggs were randomly distributed in the foam. After hatching, tadpoles tended to concentrate in the bottom of the nest, close to the water surface.

Tadpoles (Figure 1) were mainly found in the shallow parts of the tank (5–20 cm), in areas with marginal vegetation and mud on the bottom. Development in the foam nest was not synchronised, early larvae left the foam after 48 h and late individuals after 72 h, both in the laboratory and under natural conditions.

In October 1996, the first tadpoles in initial stage (25) and intermediate stages (26–39) were collected. Tadpoles in final stages (40–46) and froglets were collected in early November. During November and December, tadpoles in all larval stages were found and in mid-January the last tadpoles at the final larval stages were collected.

The minimum and maximum total lengths of tadpoles in the early stages were 6.5 and 16.4 mm (mean=11.9, SD=2.5 mm,  $n=21$ ); in intermediate stage were 15.4 and 28.4 mm (mean=20.5, SD=2.9 mm,  $n=43$ ), and in the last stage were 20.9 and 28.0 mm (mean=24.5, SD=1.6 mm,  $n=38$ ).

## Discussion

### *Breeding activity and seasonality*

The timing of the reproductive activities of *Physalaemus centralis* was clearly affected by rainfall. This factor may indicate water availability, which generally stimulates anuran reproduction (Telford and Dyson 1990; Gascon 1991). This same pattern was observed for *P. centralis* by Rossa-Feres (1997) in southern Brazil and for *P. cuvieri* in different parts of Brazil (Bokermann 1962; Cardoso 1981; Barreto and Andrade 1995). Higher atmospheric humidity favours acoustic communication because sound propagation is more efficient (Littlejohn 1977). Temperature also affects the beginning of vocalization and the maximum number of individuals calling. Concentration of the reproductive activity in the rainy period is a pattern commonly observed in *Physalaemus* species (Bokermann 1962; Cardoso 1981; Barreto and Andrade 1995, Wogel et al. 2002, Giaretta and Menin 2004).

Although *P. centralis* has a relatively long breeding season (Wells 1977), the number of individuals in activity is not constant. Males of *P. centralis* probably perceive the proximity of rain because the number of calling males increased before rainy days. This suggestion is supported by the correlation between the number of active males and mean air pressure observed in this study. Heavy rains were preceded by a slight alteration in this variable. Green (1990) concluded that temperature and rainfall influence the breeding activity of *P. pustulosus*, since there was a significant correlation between the number of individuals in the chorus and temperature and rainfall values during previous days. Although calling activity seems to be affected by rainfall, the influence of abiotic factors on the mating activity of *P. centralis* may be expected.

Sunlight influences the timing of anuran vocalization and the seasonality of the reproductive activity (Cardoso and Haddad 1992). During the breeding season, the onset of *P. centralis* vocalization was significantly correlated to sunset. The calling period which lasted approximately 6 h and ceased around 24:00 h was correlated to the number of individuals in a chorus, and not with air temperature. The same pattern was observed for an anuran community in southeastern Brazil (Cardoso and Haddad 1992). The cessation of calling activities around midnight is not totally understood, but some explanations could be: (1) to avoid loss of energy due to lower temperatures (Cardoso and Haddad 1992) and (2) to use the rest of the night for activities related to feeding.

### *Calling sites and territoriality*

Calling sites observed in this study were the same as those observed for other species in the genus (Cardoso 1981; Barreto and Andrade 1995; Wogel et al. 2002), including *P. centralis* in the southeastern (Rossa-Feres 1997) and central regions of Brazil (Bokermann 1962).

Anuran territoriality is related to intraspecific competition for limited resources such as females and sites for oviposition, calling, and feeding (Wells 1977, 1978). *Physalaemus centralis* defends small puddles, which it apparently only uses as calling and courtship sites because no male was observed feeding at these sites. Territories were mainly defended through acoustic signals (territorial and encounter calls). *Physalaemus centralis* exhibited escalated agonistic interactions that were directly related to distance between males (Wells 1977). This may allow males to give up before fighting, thus avoiding the risks and costs of physical combat (e.g. Martins et al. 1998). Territoriality is not commonly reported for species of the genus *Physalaemus*. Individuals of *P. cuvieri* move little during the night and show fidelity to the same calling site for several nights (Bokermann 1962; Cardoso 1981). Moreover, physical combat between *P. cuvieri* males has already been reported (Barreto and Andrade 1995). These facts suggest territoriality in *P. cuvieri*. Nunes et al. (2003) described male combat in *P. nattereri*, in the same mode and situation that was observed for *P. centralis* (Brasileiro 1998). Ryan (1985) observed combats in *Physalaemus pustulosus*, but the author did not consider these to be territorial. Male territoriality in *P. centralis* could promote spacing among calling males, facilitating male encounter by females and reducing the risk of disturbance by other males during the amplexus (see Wells 1977; Martins et al. 1998).

#### *Courtship and egg clutches*

The courtship behaviour of *P. centralis* is similar to that observed for other species in the genus. In *P. cuvieri*, males and females performed circular movements before the males clasped the female (Cardoso 1981). Previous contact between male and female was facultative in *P. cuvieri* (Barreto and Andrade 1995), but obligatory in *P. ephippifer* (Hödl 1990). In *P. centralis* we did not observe contact, but further observations should be done to confirm this lack of previous contact between male and female. We observed females of *P. centralis* moving along the tank and they were not intercepted by any male. This suggests that females choose their males and such a pattern has been described for some *Physalaemus* species (Ryan 1985; Barreto and Andrade 1995).

Amplectant pairs of *P. centralis* mainly moved only after calling ceased. In other species, movements accompanied by interference attempts performed by non-amplectant males have been observed (e.g. Davies and Halliday 1977). Lack of movement during amplexus could be a strategy to avoid inter- or intraspecific interactions. This same behaviour has been observed in other *Physalaemus* species. It could also be related to predator avoidance.

Construction of foam nests by *P. centralis* always occurred after the chorus activity had decreased, as it also has been observed in *P. pustulosus* (Ryan 1985). It could be a means to avoid interference by competitor males and predation because male vocalizations besides attracting females can attract the attention of predators as well (Ryan 1985). The short time of amplexus in *P. centralis* indicates that the female is ready to oviposit and the male does not need to guard her. *Physalaemus centralis* constructs foam nests in a similar way to that described for *P. ephippifer* (Hödl 1990, 1992) and *P. pustulosus* (Heyer and Rand 1977). The time spent on nest construction differs among species. Although females of *P. centralis* deposited a larger number of eggs, pairs spent less time (~30 min) to construct nests than in *P. pustulosus* (~60 min) (Heyer and Ryan 1977) and *P. ephippifer* (~40 min) (Hödl 1990).

The eggs of *P. centralis* were deposited in floating foam nests, common to other leptodactylids (Haddad and Prado 2005). Deposition of eggs and shape of foam nests of *P.*



*centralis* are similar to those of *P. cuvieri* (Bokermann 1962; Cardoso 1981; Barreto and Andrade 1995) and *P. ephippifer* (Hödl 1990). However, the mean number of eggs produced by *P. centralis* (1872) was much higher compared to that of *P. atlanticus* (81) (Haddad and Sazima 2004), *P. signifer* (273) (Wogel et al. 2002), *P. cuvieri* (474) (Barreto and Andrade 1995), and *P. ephippifer* (415) (Hödl 1990), but lower than that of *P. fuscomaculatus* (2465) (Giaretta and Menin 2004). This difference was expected because in several anuran species there is a positive correlation between clutch size and female body size (Crump 1974; Halliday and Verrel 1986). Mean SVL of *P. centralis* females (34.7 mm) is greater than that of *P. atlanticus* (22.4 mm), *P. signifer* (27.4 mm), *P. cuvieri* (31.2 mm), and *P. ephippifer* (31.0 mm), and smaller than that of *P. fuscomaculatus* (39.3 mm).

Eggs of *P. centralis* were small and larval development was relatively fast. These features are suggested to represent adaptations to temporary water bodies (Duellman 1978; Wilbur 1980). Larval development in *P. centralis* in the field was shorter than that observed in *P. cuvieri* (Bokermann 1962; Cardoso 1981; Barreto and Andrade 1995). Water depth (Seale 1980), water temperature, presence of nest and tadpole predators (e.g. Dillon and Fiaño 2000), and water chemistry (Fegraus and Marsh 2000) have all been suggested as important factors influencing rate of growth, development, and survival of tadpoles in nature.

Tadpoles of *P. centralis* were always found close to the tank edges, in shallower parts where organic material was accumulated. This pattern was also observed by Rossa-Feres (1997) for *P. centralis* and *P. cuvieri*. The combination of shallow water and vegetation may provide the tadpoles with higher food availability, and with protection against visually oriented predators inhabiting the deep parts of the tank (Diaz-Paniagua 1987).

### Acknowledgements

We thank Kelly Zamudio, Ricardo J. Sawaya, and Cynthia A. Prado for suggestions on earlier drafts of the manuscript. P. J. Hayward and two anonymous reviewers provided helpful suggestions. We also thank Katia Micchi and Renato Oliani for field assistance; Kelly Zamudio and Jeanne Robertson for help with the English version; and Norberto Hülle for help with graphs. Denise Rossa-Feres and Fausto Nomura kindly provided the tadpole photograph. CEPTA/IBAMA granted access to the study area and logistical support. We thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Pesquisa) for fellowships. Adão J. Cardoso (*in memoriam*) guided the initial part of this study. This study was supported by CAPES and FAPESP (Fundação de Apoio à Pesquisa do Estado de São Paulo).

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