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Reproductive aspects of a population of *Physalaemus gracilis* (BOULENGER 1883) (Anura: Leptodactylidae) from south Brazil

Aspectos reprodutivos de uma população de *Physalaemus gracilis* (BOULENGER 1883) (Anura: Leptodactylidae) do sul do Brasil

Schaiani Vanessa Bortolini[1]*
schai.bortolini@gmail.com

Fernanda A. Gonçalves[2]
fanzileiro@gmail.com

Noeli Zanella[2]
zanella@upf.br

Abstract

We investigated the reproductive biology of *Physalaemus gracilis* in a population from the state of Rio Grande do Sul (Brazil), and collected a total of 871 individuals through pitfall traps from May 2001 to January 2003. The largest collection of individuals occurred during the autumn and winter months, in July 2001 and from May to August 2002. The occurrence of individuals caught in pitfall traps was positively related to the temperature. The data about snout-vent length changed throughout the seasons, decreasing in spring and summer, possibly due to the recruitment of juvenile individuals. This population presented a seasonal reproductive activity and sexual dimorphism, characterized by higher values of snout-vent length and weight in females in comparison to males. The reproductive investment of the studied population supports the idea that larger females invest proportionally more in their offspring. The gradual increase of female gonadosomatic index, which coincided with the largest amount of mature oocytes, may indicate that females were prepared to oviposition process that would probably occur in spring. In contrast, males may have a continuous readiness to mate throughout the year.

Keywords: amphibians, gonadosomatic index, reproductive investment, seasonality.

Resumo

Investigamos a biologia reprodutiva de *Physalaemus gracilis* em uma população do estado do Rio Grande do Sul, Brasil. Coletamos um total de 871 indivíduos por meio de armadilhas de queda, de maio de 2001 a janeiro de 2003. O maior número de indivíduos ocorreu durante o outono e inverno, nos meses de julho de 2001 e entre maio e agosto de 2002. A ocorrência de indivíduos capturados nas armadilhas relacionou-se positivamente à temperatura. O comprimento rostro-cloacal variou ao longo das estações, diminuindo na primavera e no verão, possivelmente devido ao recrutamento de indivíduos juvenis. Essa população apresentou atividade de reprodução sazonal e dimorfismo sexual, caracterizado por maiores valores de comprimento rostro-cloacal e peso maior das fêmeas. O investimento reprodutivo da população estudada sugere que fêmeas maiores investem proporcionalmente mais em sua prole. O aumento gradual do índice gonadossomático nas fêmeas, que coincidiu com a maior quantidade de ovócitos maduros, pode indicar que as fêmeas estavam preparadas para o processo de oviposição, que provavelmente ocorre na primavera. Já os machos podem ter disponibilidade contínua para acasalar ao longo do ano.

Palavras-chave: anfíbios, índice gonadossomático, investimento reprodutivo, sazonalidade.

[1] Universidad de la República. Facultad de Ciencias. Instituto de Ecologia y Ciencias Ambientales. Laboratório de Sistemática e História Natural de Vertebrados. Iguá 4225, Montevideo 11400, Uruguay.
[2] Universidade de Passo Fundo. Instituto de Ciências Biológicas. Caixa Postal 611, 99001-970, Passo Fundo, RS, Brasil.
* Corresponding author

Introduction

The reproductive strategies of amphibians are associated with a variety of activity patterns that maximize reproductive success under particular environmental and evolutionary constraints (Duellman and Trueb, 1994). Interspecific and intraspecific variations, such as body size and specific advertisement call of each species, are the major contributing factors in the natural history of anuran amphibians, including their reproductive patterns (Dullman and Trueb, 1994; Wolgel *et al.*, 2002; Camargo *et al.*, 2005). The reproductive strategies of anurans are optimal combination of morphological, physiological, behavior and life history that maximize reproductive success under particular environmental and conditions (Jørgensen, 1992; Camargo *et al.*, 2005). Changes in temperature and photoperiod, for example, may stimulate gametogenesis in some species and set up continuous and discontinuous cycles (Jørgensen, 1992).

Reproductive effort is the proportion of resources that an organism spends for reproduction in a given period of time (Gadgil and Bossert, 1970). For anuran females without parental care, reproductive effort depends, to a great extent, on the total amount of energy stored in oocytes (Grafe *et al.*, 1992; Castellano *et al.*, 2004). These resources are known for being directly affected by factors, such as the amount of energy stored in fat bodies (Jørgensen, 1992). Owing to the large variety of activity patterns and reproductive strategies, reproductive biology can be characterized from the analysis of the gonadal developmental stage according to the season of the year. Wells (1977) characterized the temporal strategies of anuran reproduction in two types: prolonged and explosive. According to him, however, these temporal patterns are the extremes of a continuum and most species are at intermediate points of this gradient. In the prolonged breeding the species spend a lot of time choosing their partners, as there is no synchronicity in the arrival of males and females at the breeding site. In contrast, in the explosive breeding, species use a short period of time, male density is high and migration of both sexes is synchronized, with fewer opportunities for partner selection (Wells, 1977).

Some studies about reproductive biology of the genus *Physalaemus* investigated the reproductive effort, relationship between body size and fecundity, the use of fat bodies as reserves of energy for vitelogenesis, among other aspects of reproduction (Camargo *et al*., 2008; Maneyro *et al*., 2008; Pupin *et al*., 2010; Pereira and Maneyro, 2012). The genus *Physalaemus* has 47 species distributed in the Neotropical region (Frost, 2017), and some species are very well adapted to urban areas. *Physalaemus gracilis* occurs throughout South Brazil, Uruguay, Paraguay and Argentina (Frost, 2017). This leptodactylid is very abundant and inhabits both undisturbed as well as urbanized areas (Camargo *et al*., 2005). The reproduction sites are very varied, being in general, lentic water bodies, with abundant floating vegetation (Maneyro and Carreira, 2012). Thus, due to the importance of biology studies for the conservation of species, the aim of this study was to contribute to the knowledge of the reproductive biology data on the species, identifying its breeding season and describing its reproductive investment in northern Rio Grande do Sul, Brazil.

Materials and methods

Study area

We collected the specimens in a forest fragment, in Passo Fundo, Rio Grande do Sul (28º 15’ S, 52º 24’ W) (Figure 1), at an altitude of approximately 630m. The forests in the study area are typical plant formations of mixed tropical forest, represented mainly by *Araucaria angustifolia* (Bertol.) Kuntze (Araucariaceae) (Quadros and Pillar, 2002). The region is characterized by humid subtropical climate, with evenly distributed rainfall over the year and temperature of the hottest month higher than 22°C while that of the coldest month ranges from -3 to 10°C (annual average of 16.9 to 18.4°C).

Activity patterns

To analyze the patterns of activity, we collected the individuals monthly between May 2001 and January 2003 (except for July 2001 and July and September 2002) and their sex was identified by their external morphology (presence of vocal bag in males) (Silva and Rossa-Feres, 2010). We used pitfall traps with drift fences (barrels of 150 l) to sample the population (Zanella and Cechin, 2006), installing two lines of ten containers inside the forest. The traps remained open during all the study and were inspected three times a week.

Some specimens of *Physalaemus gracilis* caught in this study were deposited in the Collection of Amphibians of Universidade de Passo Fundo (CAUPF) and were used in our study for data analysis of reproductive cycle.

Reproductive cycle

We randomly chose a monthly sample of individuals from the CAUPF for reproductive cycle analysis, corresponding to March 2001 from August 2002 (except for June 2001, 2002), totaling 93 individuals. All specimens had their mass (g) and snout-vent length (SVL) determined by an analytical scale (0.0001g) and by a 0.1-mm precision caliper.

The specimens were later dissected in the ventral region and their sex was identified by gonadal assessment (Curi *et al*., 2014). The gonads from both sexes were re-

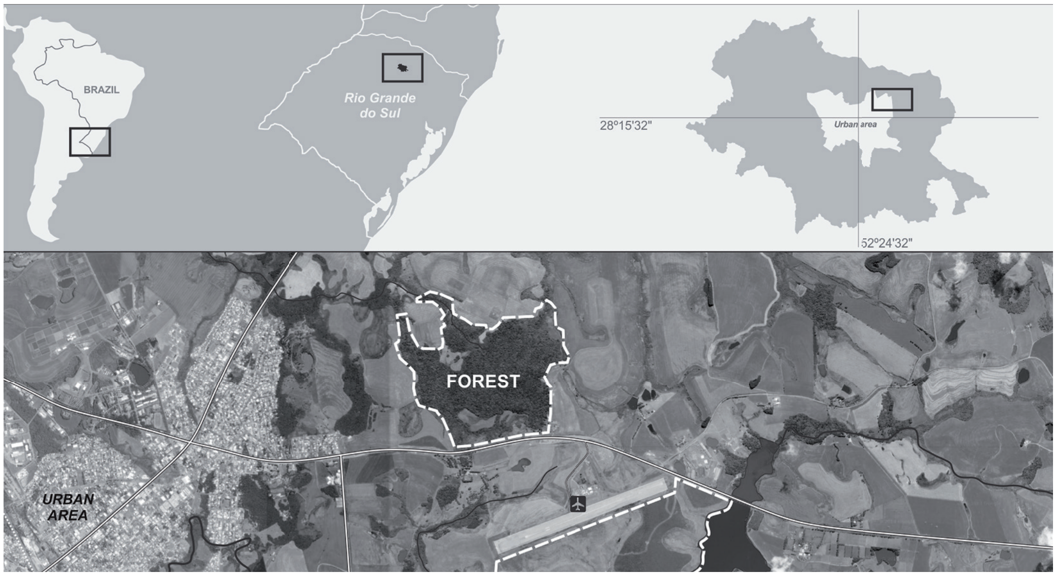

Figure 1. Location of the study area in Passo Fundo, southern Brazil.

moved in order to examine their anatomical aspects, using a stereomicroscope. In females, the gonad mass was determined using a digital balance (precision of 0.001g) and the total number of oocytes was counted. We classified the oocytes into mature, immature and atresic, according to their level of development and according to the method described by Pereira and Maneyro (2012). Mature females were those that present mature oocytes. Females with immature oocytes, with white-colored, translucent oocytes, or with signals of atresic oocytes or straight oviducts were considered immature (Camargo *et al.*, 2008; Pereira and Maneyro, 2012). We determined the reproductive investment of mature females by linear regressions between SVL and number of mature oocytes (NMO) and gonad mass (GM); and between body mass (BM) and GM and NMO. The gonadosomatic index (GSI) was calculated based on the biometric data of mature females, using the expression: GSI= (GM/BM) X100, GSI corresponding to the percentage of body mass allocated in gonad (Prado *et al.*, 2000).

We applied a similar protocol to males, the testicles of which were weighted (on an analytical scale 0.0001g) to determine the GM and the GSI. We used both GM and GSI to demonstrate the testicular activity of mature males (see Oliveira *et al.*, 2007). We considered immature those males with a vocal bag, but the testicles of which were very small and could not be weighted.

The fat bodies of females and males were weighed on an analytical scale (0.0001g) and classified into four categories (absent, scarce, middle and abundant) in order to analyze potential associations of their size with reproductive activity, considering BM and GM (Pereira and Maneyro, 2012).

Data analysis

We used the t-test to analyze the sexual dimorphism in the individuals, to compare the SVL and BM of adult females and males, as well as to calculate the mean oocytes of mature and immature females. The Spearman's correlation test was used to calculate relationship between the individuals caught and the incidence of rainfall and temperature, besides the relationship between GM and fat bodies. This analysis was performed using BioEstat 5.3 (Ayres *et al.*, 2007).

We obtained the temperature and rainfall data from a meteorological local station, 10 km away from the study area (CNPT/Embrapa, 2016).

Results

Activity patterns

A total of 871 individuals was observed during the study period (393 females, 236 males and 242 immature individuals; Figure 2), the largest number of mature individuals was found in the coldest months in June 2001 (N=187) and from May to August 2002 (N=301). Females were more abundant from April to August 2002 ($\overline{X}$=51.75; N=207). The immature individuals appeared frequently during the spring and summer (between November 2001 and May 2002; N=179). The mean SVL decreased in both seasons, representing the recruitment of the immature specimens (Figure 3). The frequency of individuals caught in pitfalls was positively correlated with temperature (R^2=0.51, p= 0.027), but not with rainfall (R^2= 0.29; p = 0.24) (Figure 4).

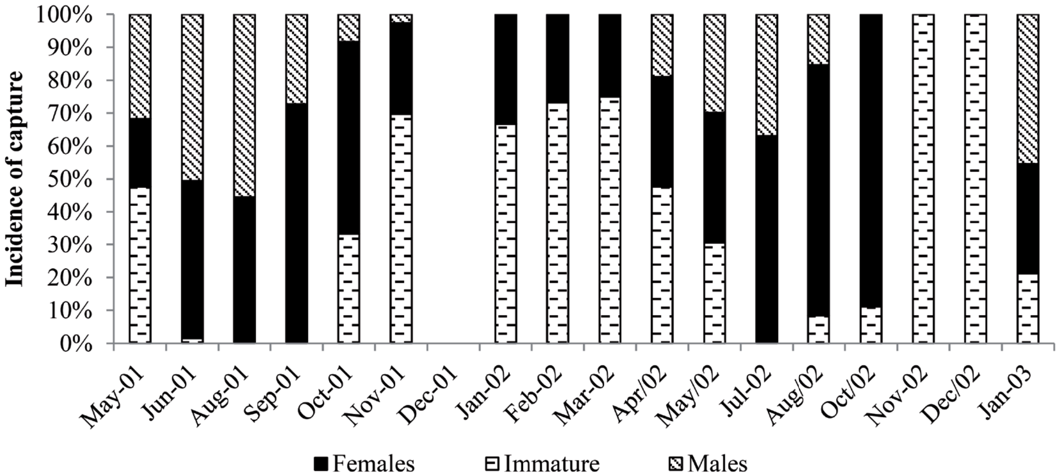

Figure 2. Monthly incidence of capture of mature and immature individuals of *Physalaemus gracilis.*

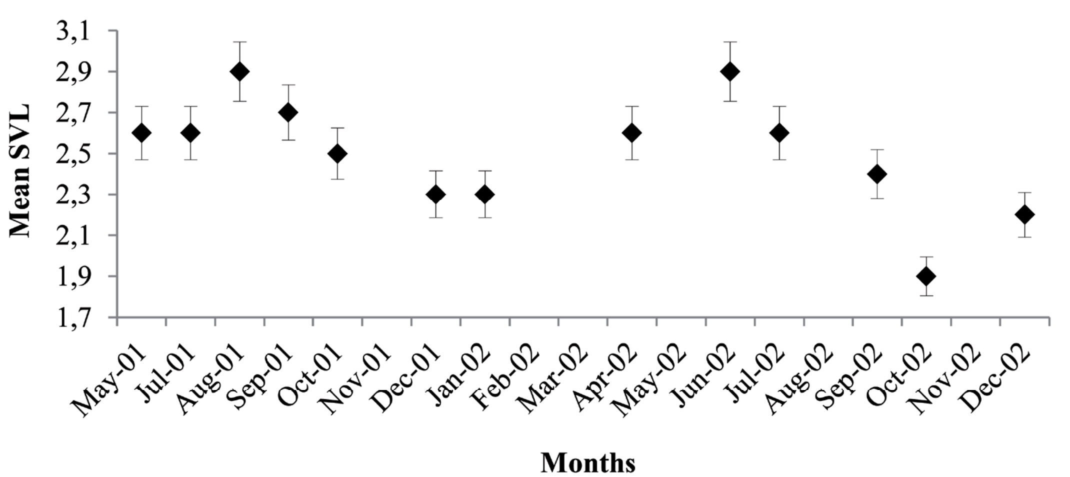

Figure 3. Monthly average variation in snout-vent length (SVL) values of *Physalaemus gracilis.* Vertical bars represent standard errors.

Reproductive cycle

From 93 individuals used to analyse the reproductive cycle, 51 were females, 35 were males and 7 of them could not be identified because they did not present gonad structures defined.

Among females, 30 were mature and 21 immature specimens. The largest number of immature females was detected in spring and summer (between September and March; N= 14) while mature females were collected in autumn and winter (between April and August; N=21), but these differences between seasons were not significant (p= 0.91). The immature females of *P. gracilis* had a mean SVL of 26.43 mm compared to 29.4 mm in mature females and the average mass was 1.40g, while that of mature ones was 1.97g. The differences in SVL and BM between immature and mature females were significant (t=3.12; p=0.004 and t=3.42; p= 0.001, respectively).

Between October and May, only females with atresic and immature oocytes were found, indicating a period for preparation and maturation of the oocytes, while the highest number of mature oocytes was observed between April and August. The number of oocytes in both ovaries of immature females ranged from 47 (female captured in February; SVL=25.8 mm) to 403 (female captured in September; SVL=33.7 mm) ($\overline{X}$=144 ± 99.9; N= 21). The minimum number of oocytes in mature females was 205, whereas the maximum number was 522 both from females captured in July (SVL=29.9 mm; SVL=30.2 mm, respectively) ($\overline{X}$=323±90.9; N=30).

The GM of immature females ranged from 0.0016 to 0.72g ($\overline{X}$=0.07 ±0.19; N=21), whereas that of mature fe-

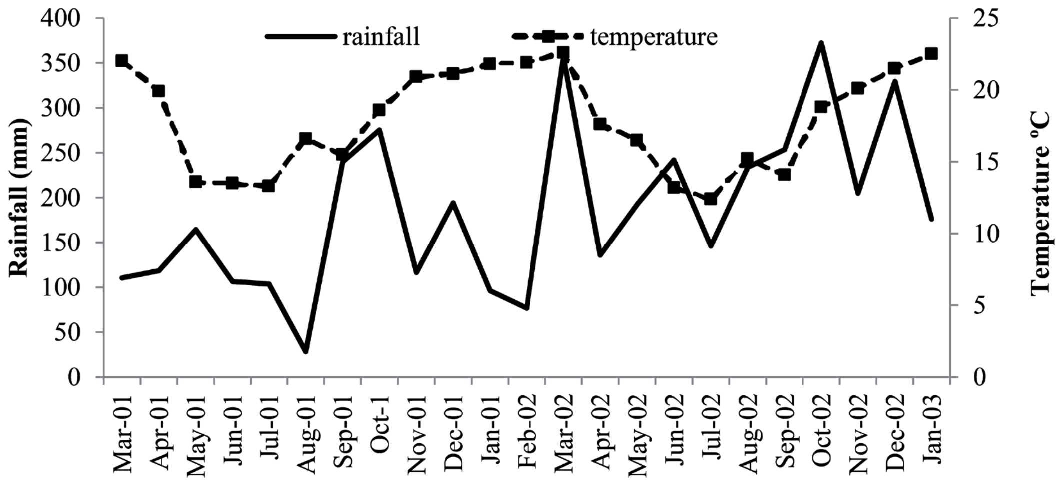

Figure 4. Monthly average temperature and rainfall accumulated in the study area between May 2001 and January 2003.

males ranged from 0.0016 to 0.93g ($\overline{X}$=0.32 ±0.2022; N= 30). There was a positive correlation between SVL and fecundity (NMO), SVL and GM, BM and GM, as well as BM and NMO (R^2=0.62; p= 0.0005; R^2=0.50; p=0.005; R^2=0.65; p=0.0003; R^2=0.78; p=0.0001, respectively) in mature females. The female GSI ranged from 0.24% to 40.96% ($\overline{X}$=14.50% ±9.84; N=30), presenting a gradual increase between May (15.9%) and August (38.3%) 2001 and a decrease in spring and even. The female GSI drastically reduced during January (0.45%) and February (0%) 2002, increasing again in the winter (Figure 5a). There were significant differences on July and August in relation to the remaining months of the year (t=5.059; p=0.0001). No significant correlation was observed between GM and relative abundance of fat bodies in females (R^2= 0.079; p=0.68) (Figure 6a).

From 35 males, 29 were adults and 6 immature specimens; all immature individuals were found between August and March. The average of SVL of immature males was 20.2 mm and that of mature males was 27.8 mm. The mean mass was 0.64g for immature males and 1.55g for mature ones. The differences in SVL and mass between mature and immature males were significant (t=5.09; p=0.0001 and t=3.90; p=0.0004, respectively). The GM of mature males varied from 0.0012 to 0.0077g ($\overline{X}$=0.0037±0.0016; N=29). The GSI ranged from 0.15% to 0.39% ($\overline{X}$=0.24% ±0.06; N=29) and the differences between the months were not significant (t=-1.07; p=0.30) (Figure 5b). Fat bodies were detected from February to August (Figure 6b). There was a significant correlation between GM and fat bodies in them (R^2= 0.44; p= 0.016). Sexual dimorphism was detected in the studied population, since females were significantly longer and heavier than males (t=2.18; p=0.03; t= 3.12; p= 0.003, respectively).

Discussion

Activity patterns

In the present study we found the largest number of mature individuals in the coldest months. The individuals present a sexual dimorphism, demonstrated by the size of females, which are longer than males, as observed in 90% of anuran species (Shine, 1979) and others Leptodactylidae (Rodrigues *et al.,* 2004; Camargo *et al.,* 2005; Pereira and Maneyro, 2012). There are multiple causes for that, such as the capacity of larger females to spawn and lay larger eggs (Crump and Kaplan, 1979; Prado *et al.,* 2000) or restraints on the growth of males due to the energy demand related to the breeding period (Woolbright, 1989).

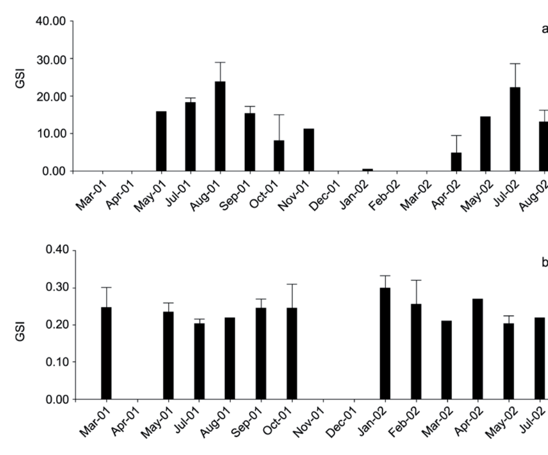

Figure 5. Monthly average of gonadosomatic index in (a) mature females and (b) mature males of *Physalaemus gracilis.*

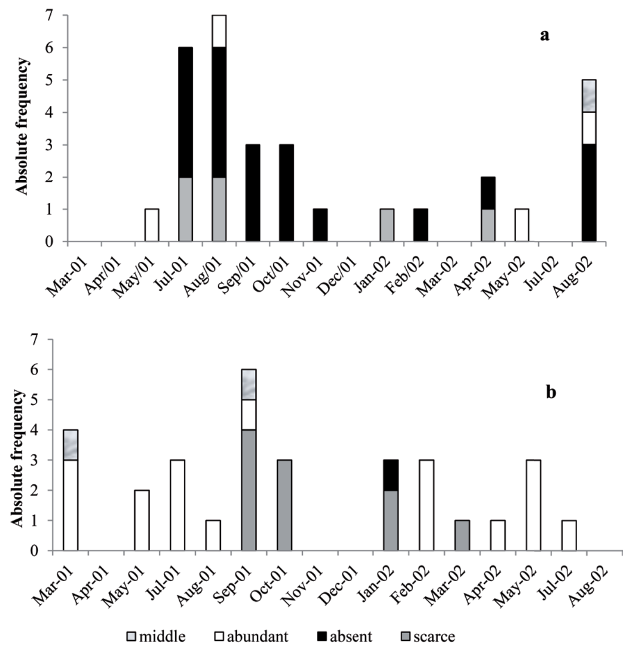

Figure 6. Distribution of fat bodies in (a) mature females and (b) mature males of *Physalaemus gracilis.*

The reproductive investment of the studied population supports the idea that larger females invest proportionally more in their offspring. The largest number of collected females occurred in the autumn and winter, which coincide with the reproductive investment of mature females of *P. gracilis*. The breeding season of this population coincides with the population of *P. gracilis* investigated by Camargo *et al.* (2005), in Maldonado, Uruguay, which is a nearby region with similar weather conditions.

The temperature, rainfall and photoperiod are the main environmental factors involved in the regulation of the reproductive cycle of amphibians, influencing in the reproductive pattern, fecundity, development, and growth of embryos (Morrison and Hero, 2003; Toledo *et al.,* 2003; Camargo *et al*., 2008, Curi *et al*., 2014; Elgue and Maneyro, 2017). The high temperature may be the main factor that regulates the reproduction of *P. riograndensis* and *Elachistocleis bicolor* in Uruguayan populations (Pereira and Maneyro, 2012; Elgue and Maneyro, 2017). A study carried out on a population of *P. gracilis* in the same country described a peak of activity in spring. Our study demonstrated that *P. gracilis* has a seasonal breeding activity, influenced by the temperature, as observed in several studies (Camargo *et al*., 2005; Maneyro *et al.,* 2008; Pereira and Maneyro, 2012; Elgue and Maneyro, 2017), and may indicate the potential of this species to breed in seasons with lower temperatures. The decreased reproductive activity of *P. gracilis* in the studied area, observed mainly in the warmest months of the year, may indicate the adaptation of this species to avoid reproduction when evaporation is presumably higher, as related by Camargo *et al.* (2005).

Reproductive cycle

Our findings indicate reduction in the average body size of the sample, with mean of SVL slower in spring and, more sharply, in summer, and that may be interpreted as recruitment of new individuals emerging from the last breeding season as observed in other studies (Giaretta and Menin, 2004; Lombardo, 2011; Oliveira *et al.,* 2007). We observed the variation of SVL at different times of the year and it may also indicate that young and adult individuals do not occur simultaneously in the study area, as verified monthly for *P. gracilis* (Camargo *et al.,* 2008) and *P. riograndensis* (Pereira and Maneyro, 2012), both in Uruguay. Camargo *et al.* (2005) noted the presence of gravid females and males in advertisement call activity, followed by a decrease in their mean body length, when young individuals were predominantly captured.

In the investigated population, the relationship between fecundity and SVL of females was significant, as observed for *P. albonotatus* and *P. nattereri* (Rodrigues *et al.,* 2004) and *P. riograndensis* (Pereira and Maneyro, 2012). Other studies demonstrate that the number of oocytes and gonad mass are positively correlated with the size of females (Pradeiro and Robinson, 1990; Prado *et al.,* 2000, Curi *et al*., 2014; Valdez and Maneyro, 2016). However, fecundity is not always directly proportional to body length, as it may be related to body mass (as verified in the present study) and to the type of reproductive investment used by each species (Bonnet *et al.,* 2003). Reproductive investment data on this population of *P. gracilis* support the idea that larger females invest proportionally more in their offspring, as observed for *P. biligonigerus* (Camargo *et al.,* 2008) and *P. riograndensis* (Pereira and Maneyro, 2012).

In many cases, reproductive investment and timing of reproduction can be predicted by gonadosomatic index. The females of the analyzed population invested 14.5% of their weight in the gonads, this rate is lower to that reported for *P. albonotatus* (27.3%) and *P. natterei* (22.2%) (Rodrigues *et al.,* 2004), but higher than that of *Fejervarya limnocharis* (12.37%) (Othman *et al.,* 2011). Often the GSI values are the highest when the amphibians are preparing for the mating (Oliveira *et al.,* 2007; Othman *et al.,* 2011). Our results showed that there was a gradual increase of female GSI between April and August. This period coincided with the largest amount of mature oocytes found, and may indicate that the females are preparing to the oviposition process during this time. The drop in female GSI and the reduction in the number of mature oocytes may be interpreted as the occurrence of oviposition in spring. No significant correlation was observed between GM and relative abundance of fat bodies in females ($p=0.68$). According to Verrel *et al*. (1986) and Ritke and

Lessman (1994), the fat bodies likely have a minimum size and even disappear during the breeding season, decreasing progressively as the size of oocytes increases (Frost, 1983; Jørgensen, 1992). In females of *P. riograndensis*, the fat bodies were correlated with the gametogenic activity (Pereira *et al*., 2015). Therefore, as observed in this study the distribution pattern of fat bodies suggests that they can be used as a resource for vitellogenesis in winter.

According to Oliveira *et al.* (2007) and Othman *et al.* (2011), the high GSI and GM values indicate high testicular activity. *Dendropsophus minutus* (Oliveira *et al*., 2007) presented increase of GM and GSI in October, showing only a peak of testicular activity, as observed for *Rana dybowskii* (Ko *et al*., 1998). For *F. limnocharis* (Othman *et al*., 2011) a cyclic activity on GSI was evidenced, where the index was lower during the driest months and gradually increased in the months immediately preceding the rainy season. This pattern also could be insinuated for *Xenopus tropicalis* (Olmstead *et al*., 2008). Comparing to female GSI, the male GSI did not show a similar trend, with gradual increase in females in the autumn and winter. The GSI of males from this study did not present significant differences during the months of the year and maintained relatively constant rates. It could represent a high reproductive development and a continuous readiness for males to mate throughout the breeding (Othman *et al*., 2011). Although the spermiogenesis is maintained throughout the year, only part of the mature spermatozoa coincides with the female reproductive period (Díaz-Páez and Ortiz, 2001). In males, there is a correlation between an increase in the testicle size and the presence of fat bodies. Gonadal function in the males is less dependent on nutritional conditions than females, suggesting that fat bodies act on the reduction of gonadotropin secretion (Jørgensen, 1992).

In conclusion, this study indicates that the population of *Physalaemus gracilis*, of this study area, has a seasonal breeding activity, with variations influenced by temperature. The gradual increase of female GSI (in winter), which coincided with the largest amount of mature oocytes, may indicate that females were prepared to oviposition process, that would probably occur in spring. In contrast, males may have a continuous readiness to mate throughout the year. Despite of being an abundant species and present a large geographic distribution, factors such as habitat fragmentation and environmental temperature changes, could represent a threat to the populations of *P. gracilis*. Therefore, we emphasize the importance of the implementation of conservation strategies in fragmented environments, as well as new studies about the biology of these amphibians.

Acknowledgments

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