

Size-fecundity relationships and reproductive investment in females of *Physalaemus riograndensis* Milstead, 1960 (Anura, Leiuperidae) in Uruguay

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We describe the reproductive biology of female *Physalaemus riograndensis* from Uruguay. Mature ovaries with post-vitellogenic oocytes were extracted from females to obtain an index of reproductive investment (RI). Monthly variation in snout-vent-length (SVL, $n=328$) suggests a seasonal variation in reproductive activity. Gravid females were found between October and April. RI values were generally lower than RI in other species in the genus. There was a positive correlation between SVL and fecundity for both linear and exponential models. Relative size of fat bodies was highest during the period of highest reproductive activity, suggesting that they retain energy for vitellogenic activity. The reproductive dynamics of *P. riograndensis* resembles one of prolonged breeding anurans.

Key words: anura, body size, fat body, fecundity, *Physalaemus riograndensis*, reproductive investment

INTRODUCTION

Amphibians exhibit a wide diversity of activity patterns and reproductive strategies that correlate with prevailing weather conditions together with other factors (Bevier, 1997). In temperate regions, where seasonality is strongly marked, reproductive activity of amphibians depends on a combination of abiotic factors that includes temperature and rainfall, showing an annual pattern (Wiest, 1982; Díaz-Paniagua, 1986; Rome et al., 1992; Pombal, 1997; Canavero et al., 2008; Both et al., 2008). Seasonal variation could affect gametogenesis as well as regulate the length of the reproductive season (Jørgensen, 1986), whereas rainfall could be involved in the mechanism that activates the beginning of the reproductive event (Stebbins & Cohen, 1995).

Reproductive effort is the proportion of resources that an organism spends for reproduction in a given period of time (Gadgil & Bossert, 1970). For anuran females that do not exhibit any parental care, reproductive effort is reflected by the total amount of energy spent in oocytes (Castellano et al., 2004), which can be affected by measures of female condition such as the amount of energy stored in fat bodies (Jørgensen, 1992). The weight of fat bodies varies according to annual temperature and food availability (Church, 1960; Frost, 1983; Jørgensen, 1986), and represents a good indicator of the nutritional state of an organism (Jørgensen, 1992).

The size of fat bodies has been shown to influence gametogenesis in several anurans (Long, 1987). A further central role in the allocation of energy to a given clutch is the size of a female. A positive correlation between body size and clutch size has been shown repeatedly (Castellano et al., 2004; Rodrigues et al., 2004; Camargo

et al., 2005; 2008; Maneyro et al., 2008). However, relationships between oocyte and female sizes can also be negative (Jørgensen, 1992).

The genus *Physalaemus* belongs to the family Leiuperidae, and in Uruguay is represented by six species (Maneyro & Beheregaray, 2007). Studies carried out on Uruguayan populations of *P. gracilis* and *P. biligonigerus* have shown that reproductive activity of these species is seasonal (Camargo et al., 2005; 2008), and triggered by the heavy rainfall occurring between September and March (Langone, 1995; Achaval & Olmos, 2003). The influence of rainfall on reproductive activity has also been shown for *P. henselii* (Maneyro et al., 2008) and *P. centralis* (Brasileiro & Martins, 2006).

We report on aspects of reproductive activity of females in a population of *P. riograndensis* in the Pampa Biome, Uruguay (Núñez et al., 2004; Frost, 2010). Our aims were to (i) assess seasonal variation in the reproductive activity, and test whether fecundity correlates with body size in females, (ii) investigate the variation in reproductive investment in gravid females and analyze potential associations between fat bodies and reproductive activity of males and females. We hypothesize that if fat bodies are relevant for vitellogenic activity, a negative relationship between fat bodies and the development of oocytes will exist.

MATERIALS AND METHODS

All specimens were already available at the Zoology of Vertebrates Collection (ZVCB) of the Faculty of Sciences, Universidad de la República, Montevideo, Uruguay. Individuals were captured between March 2007 and February 2008 in the Departement of Tacuarembó (32°26'06" S, 55°31'59" W), manually and using pit-fall

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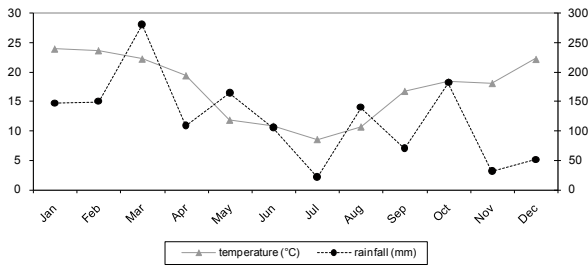


Fig. 1. Average monthly temperature and rainfall accumulated in the study zone during the period of work.

traps. Data on temperature and rainfall were obtained from the Dirección Nacional de Meteorología (National Meteorology Office) (INE, 2009; Fig. 1).

All individuals were sexed via gonadal examination and snout-vent-length (SVL) was measured in mm. The period of reproductive activity of females was determined from the degree of development of the gonads. Females were considered gravid only when they had mature ovaries and fully-developed, brown oocytes (Camargo et al., 2005). Females with whitish, translucent or atresic oocytes (seen as dark blotches in the ovaries), were considered as immature. The size (in mm) of the smallest gravid female was used as a threshold to distinguish between adults and juveniles.

Mature ovaries with post-vitellogenic oocytes were extracted and the total mass of the ovaries was measured with a digital balance (Sartorius BP 221s with 0.1 mg precision). The ovisacs were opened to separate and count the total number of oocytes (a measure of fecundity) from both ovaries (Camargo et al., 2005). We performed a Kruskal-Wallis test to assess monthly variation in the number of oocytes in gravid females.

The size of oocytes was calculated as ovary mass divided by the number of eggs. Relationships between SVL, oocyte size and fecundity were explored by linear and exponential regressions. Individual reproductive investment was calculated as the ratio between ovary mass and SVL (Camargo et al., 2005). The software SMATR ver 2.0 was used to calculate the slope of the allometric regression (SMA) between body mass and ovary mass. Fat bodies were classified into four categories (absent, small, intermediate and large) in order to analyze potential associations of their size with reproductive activity. They were considered “large” when occupying a considerable part of the abdominal cavity, almost completely covering the gonads. “Intermediate” fat bodies only covered the front part of the gonads, and “small” fat bodies were very difficult to find.

RESULTS

Activity patterns

Most animals were between 14 mm and 17 mm in SVL, which ranged between 8 and 24 mm (mean=16.1, SD=3.09). Gravid females were found from October to April. Two peaks of abundance (March and December) were observed. There was no significant monthly variation in fecundity of gravid females ($H=5.87$, $p=0.44$, Fig. 2). The smallest gravid female was 17.1 mm in SVL.

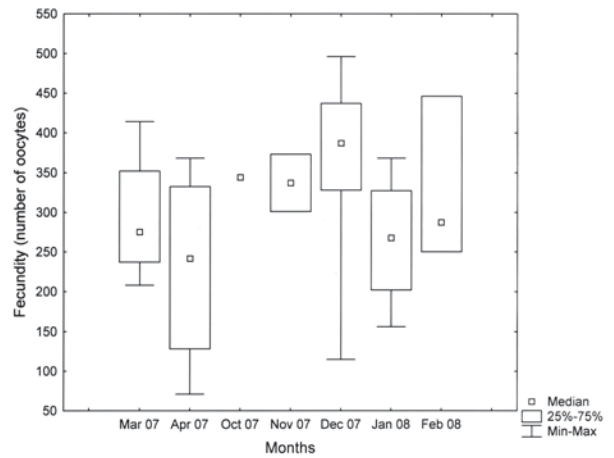


Fig. 2. Monthly variation in fecundity (number of oocytes) in gravid females of *Physalaemus riograndensis*. The points represent median values, the wide bars are the quartiles 25% and 75%; and the vertical bars are the distribution ranges.

The frequency of gravid females was positively correlated with the average monthly temperature ($R^2=0.50$; $p<0.05$), but not with monthly cumulative rainfall ($R^2=0.18$; $p=0.68$).

Female sexual cycle and reproductive investment

During the course of the study 32 mature females were found, weighing 0.91 ± 0.26 g (0.45–1.63 g) and possessing an SVL of 19.53 ± 1.44 mm (17.10–22.04 mm). The average number of mature oocytes in both ovaries was 312.75 ± 100.99 ; the minimum was 71 in a female captured in April (SVL=18.30 mm) and the maximum was 496 in a female captured in December (SVL=19.93 mm). SVL and fecundity were positively correlated for both the linear ($R^2=0.35$; $p<0.001$, Fig. 3) and the exponential model ($R^2=0.41$; $p<0.001$, Fig. 4). The relationships between SVL and egg size was significant ($R^2=0.36$; $p<0.001$), and between ovary mass and body mass ($b=2.328$, $R^2=0.623$, $p<0.001$; the slope of this regression is hyperallometric because it is significantly higher than 1) were significant. RI in gravid females averaged 0.0057 ± 0.003 (0.001–0.013) g/mm (Fig. 5).

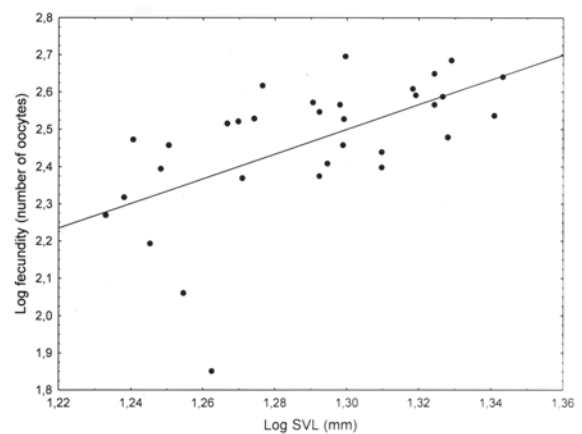


Fig. 3. Regression between snout-vent-length (mm) and fecundity (number of oocytes) in a population of *Physalaemus riograndensis*. Linear model.

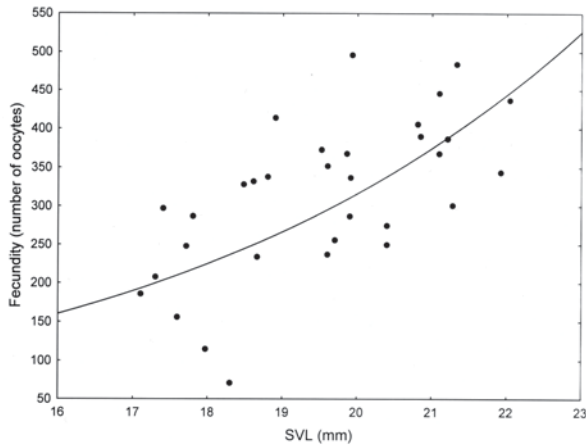


Fig. 4. Regression between snout-vent-length (mm) and fecundity (number of oocytes) in a population of *Physalaemus riograndensis*. Exponential model.

Dynamics of the fat bodies

In young individuals, no temporal variation in the size of fat bodies was observed. When young were the most common class (July and October), the majority of individuals possessed fat reserves (Fig. 6A). An increase in fat bodies can be observed for females during October, whereas in March and April fat bodies were regularly absent (Fig. 6B).

DISCUSSION

Seasonality of reproduction

Only young specimens were found between May and September, when temperatures are low. Between October and April, gravid females predominate, with one peak in December and a further peak in March. Several studies had shown that female anurans can produce more than one clutch per season (Crump, 1974; Lemckert & Shine, 1993). Prado & Haddad (2005) observed that frogs in SE Brazil show several reproductive events throughout the year. The bimodal pattern observed in *P. riograndensis* is however rather exceptional among frogs from temperate environments (Camargo et al., 2008). Females might have no resting period after their first oviposition, and retain mature eggs for a second oviposition (for example *P. thaul*, Díaz-Paez & Ortiz, 2001). The bimodal pattern could also be interpreted as different cohorts of females with earlier and later reproduction.

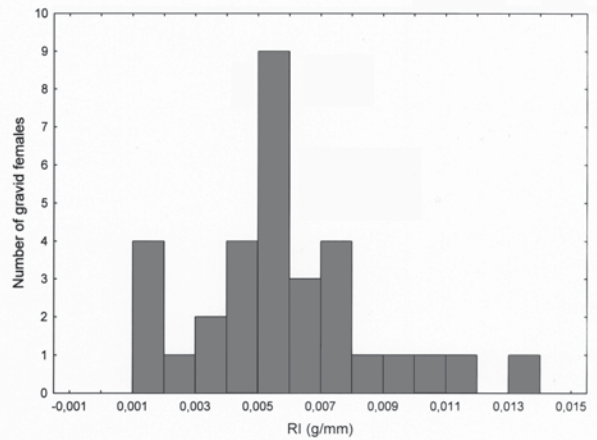
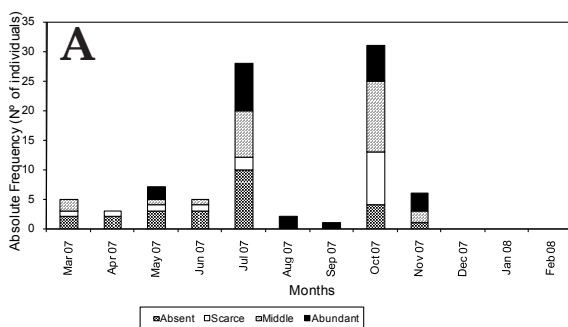


Fig. 5. Distribution of frequency of the reproductive investment of all gravid females of *Physalaemus riograndensis* collected during the period of study.

The presence of gravid *P. riograndensis* during the warm season outlines the seasonal reproductive activity of this species. Previous studies have demonstrated that some *Physalaemus* species are reproductively active during the cold season. This pattern is rather uncommon in anurans from Uruguay and from neighbouring regions, and has only been observed in some Leiuperid frogs such as *P. henselii* (Barrio, 1964; Maneyro et al., 2008), *P. fernandezae* (Barrio, 1964) and *Pleurodema bibroni* (Barrio, 1977). Monthly variation in SVL suggests that young and adults did not co-occur at the same time in the study site. Females found in July were smaller than those found in December and March, a pattern already documented for *P. gracilis* (Camargo et al., 2008).

Environmental factors and reproductive activity

In amphibians, seasonal development and gonadal activity are under control of the adenohypophysis, whose secretions are correlated to environmental changes that act as primary stimuli (Duellman & Trueb, 1994). Both temperature and activity patterns showed cyclic circannual variation. Temperature could be one of the main factors regulating reproductive activity in *P. riograndensis*, whereas cumulative rainfall was not significantly correlated with reproductive activity (as demonstrated for other anuran populations from temperate environments; Wiest, 1982).

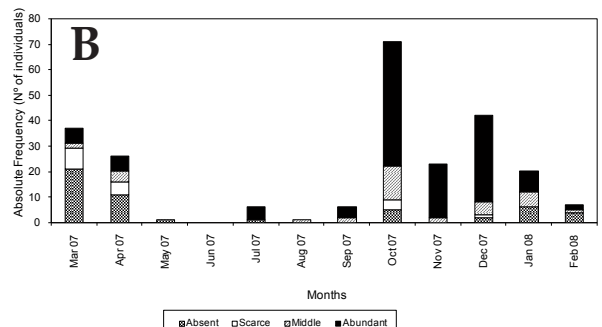


Fig. 6. Distribution of the fat bodies in (A) young females, (B) adult females of *Physalaemus riograndensis*.

In assemblages of temperate anurans, photoperiod plays an important role in aestivation, spermatogenesis and vocalization (Both et al., 2008), and can serve as a proxy for further environmental variables. Taking these facts into account, neither temperature nor rainfall exclusively regulate the period of reproductive activity of *P. riograndensis*. However, in *P. centralis*, reproductive activity was clearly affected by rainfall as well as temperature (Brasileiro & Martins, 2006). The influence of climate on reproductive activity might depend on the phylogenetic position and the reproductive dynamics within the range of explosive and prolonged breeders (*sensu* Wells, 1977).

Reproductive investment

Females invest a large amount of resources on offspring, with a trade-off between offspring quantity and fitness (Camargo et al., 2008). Females control the fitness of their offspring either by the assignment of energy and/or the genetic quality of males they mate with (Castellano et al., 2004). We found a positive and significant correlation between the number of oocytes and SVL, as has been observed in other Leiuperid anurans (Camargo et al., 2005; Camargo et al., 2008; Castellano et al., 2004; Brasileiro & Martins, 2006; Rodrigues et al., 2004). Although females of *P. henselii* and *P. gracilis* are larger than *P. riograndensis*, the average number of eggs produced is very similar (296.9, 311 and 312.7 respectively; Pupin et al., 2010). With regard to body size, females of *P. riograndensis* resemble *P. atlanticus*, *P. crombei* and *P. spiniger* (Pupin et al., 2010). Despite having similar sizes, these species have a lower number of eggs, which could be related to their more terrestrial lifestyle. In anurans, the trade-off between fecundity and egg size is more pronounced in terrestrial breeders that produce large eggs and small clutches (Pupin et al., 2010). *Physalaemus riograndensis* exhibits reproductive mode 11 following Salthe & Duellman (1973) and Haddad & Prado (2005), common with *P. henselii* and *P. gracilis*. However, the number of eggs seems to be independent from reproductive strategy in the genus *Physalaemus* overall (*P. henselii* and *P. riograndensis* are characterized by breeding during the cold and warm season, respectively, Maneyro et al., 2008).

In species such as *P. gracilis* and *P. biligonigerus*, bigger females invest proportionally more into their offspring (Camargo et al., 2008). In this study, a small number of gravid females had very high value of reproductive investment, which was also observed in *P. gracilis* (Camargo et al., 2005). Long reproductive periods make individuals more vulnerable to predators, and predation risk might be an important factor associated with reproduction in terms of survival (Camargo et al., 2008). Castellano et al. (2004) proposed that the relationship between egg size and body size in female anurans is not as marked as the relationship between clutch size and body size, due to a negative correlation between egg size and clutch size. However, in *P. riograndensis* there is a positive correlation between SVL and egg size, suggesting that females of this species invest in both quantity and quality of eggs.

Dynamics of fat bodies

In *P. riograndensis*, growth can be considered as investment in future reproduction: bigger females produce more eggs than smaller (probably younger) females due to more space in their abdominal cavity (Camargo et al., 2005). Mature females appear to refrain from feeding during reproduction (personal observation), and a full stomach would reduce the space available for eggs. However, the stomachs of all immature females collected together with females contained food items, reinforcing the idea that adult females reduce trophic activity when the ovaries are mature.

A relationship between fat bodies and gametogenesis has been recorded for several species. In temperate regions, fat bodies are used as nutritional reserves during hibernation (Prado & Haddad, 2005). There was no clear pattern of temporal variation in the size of fat bodies in young males and females, although visible fat bodies were most often observed in July and October. This suggests that individuals can use fat bodies as a source of energy for growth.

The majority of adult females (specimens collected between October and December) possessed large fat bodies, whereas from January onward individuals with no reserves at all were recorded. Fat bodies thus might contribute to the development of oocytes, with females in need for feeding before entering the reproductive season to replenish reserves. These observations agree with Díaz-Paéz & Ortiz (2001), who found that fat bodies are smaller during the spawning period, decreasing progressively as the size of the oocytes increases. Therefore, fat bodies could be used as reserves of energy at the beginning of vitellogenesis.

The results of this study indicate that female *P. riograndensis* are characterized by exhibiting a seasonal reproductive activity with individual variation in reproductive investment. Small females may spend part of the surplus energy for growth, whereas investment in more eggs becomes more beneficial than reaching larger bodies in large females. As the size of the body cavity is directly related to female fecundity, growth can be considered as an investment for future reproduction.

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APPENDIX

List of specimens of *Physalaemus riograndensis* used in this study. All specimens were collected between March 2007 and February 2008 in Puntas de Zamora (Department of Tacuarembó, Uruguay). References: ZVCB=Vertebrate Zoology Collection of Facultad de Ciencias, Universidad de la República, Uruguay.

Date	ZVCB										
Mar 07	12464	12494	12529	12553	12559	12608	12609	12610	12612	12652	12683
	12684	12685	12700	12701	12752	12755	12848	12861	12877	12899	12914
	12917	12918	12938	13010	13011	13023	13028	13033	13093	13120	13121
	13122	13127	13261	13342	13349	13359	13362	13363	13369		
Apr 07	13404	13417	13462	13463	13495	13496	13497	13521	13524	13528	13550
	13645	13646	13671	13672	13686	13740	13743	13974	13975	13976	13977
	13979	13999	14000	14026	14042	14048	14052				
May 07	14074	14123	14124	14128	14168	14238	14261	14317			
Jun 07	14605	14643	14648	14649	14650						
Jul 07	14695	14706	14707	14708	14709	14710	14711	14714	14796	14801	14802
	14810	14826	14827	14833	14836	14841	14844	14849	14871	14885	14886
	14888	14890	14905	14906	14931	14938	14942	14949	14960	14977	14982
	15040										
Aug 07	15091	15111	15170								
Sep 07	15224	15225	15227	15230	15233	15237	15278				
Oct 07	15396	15400	15403	15419	15440	15453	15459	15460	15461	15485	15493
	15494	15496	15497	15498	15502	15504	15505	15512	15514	15517	15519
	15522	15524	15526	15529	15530	15531	15532	15533	15537	15539	15541
	15542	15551	15552	15554	15556	15559	15560	15561	15562	15567	15568
	15575	15578	15580	15581	15582	15585	15587	15588	15595	15596	15597
	15598	15599	15600	15601	15603	15604	15610	15611	15616	15622	15632
	15634	15640	15641	15642	15649	15651	15656	15660	15671	15673	15674
	15676	15677	15678	15679	15680	15681	15682	15683	15687	15689	15696
	15698	15702	15706	15708	15713	15714	15715	15717	15730	15736	15738
	15748	15753	15755								
	Nov 07	15963	15964	15965	15971	15972	15978	15979	15980	15981	15983
16000		16003	16004	16010	16029	16030	16042	16049	16051	16069	16070
16081		16094	16095	16098	16104	16111	16128				
Dec 07	16212	16244	16250	16266	16267	16268	16270	16273	16278	16279	16281
	16287	16288	16290	16291	16304	16305	16306	16307	16314	16318	16353
	16360	16366	16375	16399	16409	16479	16486	16507	16508	16509	16513
	16520	16553	16554	16555	16567	16582	16585	16604	16605		
Jan 08	16629	16644	16678	16682	16683	16684	16685	16687	16695	16706	16784
	16811	16832	16845	16847	16848	16850	16872	16873	16874		
Feb 08	16920	16982	17038	17043	17045	17051	17061				