Seasonal activity and the body size–fecundity relationship in a population of *Physalaemus gracilis* (Boulenger, 1883) (Anura, Leptodactylidae) from Uruguay

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Two key life history attributes are temporal reproductive patterns and inter- and intraspecific variation in clutch size. In this work, we collected individuals of *Physalaemus gracilis* in Espinas stream (Maldonado, Uruguay) to analyze the correlation of activity with environmental variables and to evaluate the influence of reproductive investment on the body size-fecundity (BSF) relationship. In contrast to previous reports, the reproductive activity peaked in spring and was not correlated with temperature or rainfall in either sex. Regression analysis showed that the BSF relationship varied with size, and that larger females had a steeper slope than smaller females. Reproductive investment data indicate that smaller females invested less energy in their offspring than larger females, which is probably due to the imposed cost of continued growth.

**Introduction**

The reproductive strategies of anurans are optimal combinations of morphological, physiological, behavioral, and life history traits that maximize reproductive success under particular environmental and evolutionary constraints (Duellman & Trueb 1994). Two key life history attributes related to reproductive strategies are seasonal reproductive patterns and inter- and intraspecific variation in clutch size (e.g., Salthe & Duellman 1973, Crump 1974, Kuramoto 1978, Wake 1982). Whereas a bulk of data have accumulated on life history of frogs from temperate areas in the northern hemisphere, a similar knowledge for frogs in tropical and temperate regions of South America is far behind (see Donnelly et al. 2005). The temporal reproductive pattern of anurans is continuous in tropical non-seasonal climates, and seasonally cyclic in tropical and temperate regions that have a marked dry or
cold season (Duellman 1978, Jørgensen 1992). In temperate regions, the seasonal increase in temperature plays a permissive role in the activation of gonads, and regulates the duration of the breeding season, whereas rainfall often triggers the onset of the breeding season (Stebbins & Cohen 1995). While these general patterns hold for the majority of anuran species in temperate regions, variation in other factors (e.g., internal rhythms) can cause some species to reproduce for longer or shorter periods than expected (Wells 1977, Brown & Shine 2002, Morrison & Hero 2003).

Regarding intraspecific variation in the body size–fecundity (BSF) relationship, most studies report a significant and strong correlation between body size and clutch size (Tejedo 1992, Gilbert et al. 1994, Kusano & Hayashi 2002, Wogel et al. 2002). However, other studies have not found such a consistent pattern, at least for some species (e.g., Duellman & Crump 1974, Elmberg 1991, Tarkhnishvili 1993, Buschmann 2002, Tsiora & Kyriakopoulou-Sklavounou 2002). One hypothesis for these contradictory results is that females vary in the amount of energy allocated for different activities (reproductive or otherwise). This may constrain clutch size and, consequently, affect the BSF relationship (Duellman & Trueb 1994). In this sense, Bonnet et al. (2003) found that fecundity correlates well with body size in high reproductive investment (RI) females. However, when females with increasingly lower RI are included in the analysis the strength of the BSF relationship weakens.

Herein, we investigated seasonal variation in the activity of male and female Physalaemus gracilis in a population from southern Uruguay. We chose P. gracilis as the focal species because it is one of the most abundant frogs in southern Uruguay (Nuñez et al. 2004). In addition, this leptodactylid frog inhabits both undisturbed as well as urbanized areas, where foam nests are placed among the aquatic vegetation in shallow swamps and flooded areas (Langone 1995, Maneyro et al. 1995, Achaval & Olmos 2003). Previous reports indicate that the seasonal breeding activity of P. gracilis occurs after heavy rains from September to March (Langone 1995, Achaval & Olmos 2003).

Materials and methods

Study site and specimen collection

We collected P. gracilis along Espinas stream (34°47´S, 55°22´W, Departamento de Maldonado, Uruguay) between September 1998 and April 2000. The 2-km-long Espinas stream is dammed before flowing into the La Plata River and becoming a shallow, lentic area at sea level. Frogs were collected monthly in pitfall traps placed at about two meters from the stream border and remaining operative during the entire study period. Specimens were deposited in the Vertebrate Zoology Collection, Facultad de Ciencias, Universidad de la República (ZVCB). For all specimens we measured total snout-vent length (SVL) using a 0.005 cm precision digital caliper.

Patterns of activity

Activity patterns were described by considering monthly variation in the number of captures and body sizes. The period of reproductive activity of females was determined from the presence of gravid females with mature ovaries containing fully developed oocytes. The period of male reproductive activity was estimated during fieldtrips (see below), using the relative abundance of calling males based on the following ranking: (1) occasional: one male, (2) rare: two males, (3) common: three or more distinguishable males, and (4) chorus: many countless calling males (Moulton et al. 1996, Shirose et al. 1997, Driscoll 1998). Fieldtrips were conducted each month (except for February 1999 and December 1999) for two consecutive nights, between sunset and 01:00. The relative abundance of calling males was recorded at seven sites. For each month, we summed ranks over all sites to obtain an estimate of male abundance across the study area. Correlation between the reproductive activity of males and females was estimated using non-parametric Spearman’s rank test (Zar 1999). In addition, we obtained mean monthly records of temperature and accumulated rainfall from the Carrasco International Airport Weather Station, which is at sea level and near
to the coast (60 km west of the study site). We calculated non-parametric Spearman’s rank correlations between each climatic variable and the reproductive activity of each sex.

**Body size–fecundity relationships and reproductive investment**

The mature ovaries with post-vitellogenic oocytes were extracted from all gravid females, and the total mass of ovaries was measured with a digital balance with 0.01 g precision. Subsequently, ovisacs were removed to release mature oocytes and their total number (i.e. fecundity) was directly quantified for each ovary. The BSF relationship was evaluated through regression analysis, testing both linear and exponential models. In addition, we fit a pair-wise regression to analyse the potential existence of a breakpoint in the BSF function. Reproductive investment of each individual was estimated as the ratio between ovary mass and SVL.

**Results**

**Patterns of activity**

A total of 275 specimens were collected during the study period. Peaks in capture number occurred during the warm months (October–February) of each year. The body size distribution had two peaks, with modal values of 1.60 and 2.90 cm (Fig. 1).
1a). Since the minimum SVL for a gravid female (2.46 cm) corresponds to the valley of this bimodal distribution, peaks probably represent adult and juvenile individuals. The monthly variation in mean SVL indicates that adults and juveniles did not necessarily occur in the study site at the same time. Adult individuals appeared first (October–December) and then the juveniles arrived abruptly (December–January), causing a marked drop in mean SVL during both years (Fig. 1b).

The reproductive activity of both sexes was highly correlated \( r_s = 0.77, n = 17, P < 0.001 \) due to the synchronous presence of calling males and gravid females in October and November of both years (Fig. 2a). However, male activity started earlier than female activity, which was characterized by a sudden occurrence and disappearance of gravid females in the study area. Although the number of gravid females in November 1998 \( (n = 8) \) was lower than in November 1999 \( (n = 32) \), the proportion was very similar (62% and 55%) in relation to the total number of captures during these months \( (n = 13 \text{ and } 58, \text{ respectively}) \).

Neither monthly variation in temperature nor rainfall was correlated with female activity (temperature: \( r_s = 0.28, n = 19, P = 0.25 \); rainfall: \( r_s = -0.41, n = 19, P = 0.08 \); Fig. 2b) or male activity (temperature: \( r_s = -0.02, n = 18, P = 0.95 \); rainfall: \( r_s = -0.06, n = 18, P = 0.83 \); Fig. 2b). Repro-
Reproductive patterns in *Physalaemus gracilis*

Reproductive activity was not correlated with changes in temperature or rainfall that occurred in the previous month for either sex, but we did observe significant associations with the temperature one month in advance ($r_s = 0.62$, $n = 18$, $P < 0.01$ for females; $r_s = 0.52$, $n = 17$, $P < 0.05$ for males).

**Reproductive investment and body size–fecundity relationships**

A total of 64 gravid females were collected and analysed. A significant correlation between fecundity and SVL was found, and both the linear ($r = 0.60$, $F_{1,62} = 35.43$, $P < 0.001$) and the exponential ($r = 0.61$, $F_{1,62} = 5.93$, $P < 0.001$) model, had a similar fit to the data. Pairwise regression explained 72.4% of the total variance (nearly twice the variance explained for the above mentioned models), and suggests the existence of a breakpoint in SVL at 2.98 cm (Fig. 3). Females with larger SVL had a steeper slope ($b = 360.3$, SE = 137.0, $n = 30$) than smaller females ($b = 206.6$, SE = 78.1, $n = 34$). RI reached a mean value of 0.10 (SE = 0.006), ranging from 0.02–0.23 g cm$^{-1}$. In addition, the RI frequency distribution showed that most females presented lower than average RI values.
with a long tail of few females showing very high RI (Fig. 4).

Discussion

Activity patterns

Annual and seasonal variation in species activity has profound implications on ecology, from the organism level (e.g., Jørgensen 1992) to the community level (e.g., Schoener 1974, Sandvik et al. 2002). Activity periods govern the length of the breeding season and also influence the timing of reproductive bouts and the number of clutches produced in a season (Bull & Shine 1979). In addition, the type of male competition for mates and female mating preferences greatly depend on the temporal pattern of reproduction and operational sex ratios (Eggert & Guyétant 2003).

Results obtained in the present work indicate that \( P. \) gracilis activity peaks were restricted to two or three months during the warm season (spring and summer). In both years, the presence of gravid females and male calling activities peaked from October to November (or December), and subsequently, a marked decline in mean body size occurred when juveniles dominated the captures. A similar pattern of body size variation was found in \( P. \) cf. fuscomaculatus and is interpreted as the recruitment of new individuals emerging from the last breeding season (Giaretta & Menin 2004).

The phenology of \( P. \) gracilis in Uruguay corresponds to that of an early breeder, with a pattern that is characteristic of prolonged reproduction \((\textit{sensu} \text{ Wells} 1977)\). The early arrival of males to form large aggregations and the emission of calls different from the specific advertisement call (A. Camargo pers. obs.) suggest that \( P. \) gracilis males spend considerable time in chorus formation. Prolonged breeders, in contrast to explosive breeders, reproduce in permanent sites, where males tend to form lek-like, spatially structured choruses and call antiphonally to minimize male-to-male interaction (Stebbins & Cohen 1995). In this sense, intensive social interactions, including male competition and female mate selection, have been reported for other \( \textit{Physalaemus} \) species (e.g., \( P. \) cuvieri Barreto & Andrade 1995, \( P. \) pustulosus Bosch et al. 2000, \( P. \) signifer Wogel et al. 2002, \( P. \) enesefae Tárano & Herrera 2003). Nevertheless, our results contrast with previous accounts of the phenology of \( P. \) gracilis in Uruguay, where a more extended breeding period was reported (Langone 1995, Achaval & Olmos 2003). These discrepancies may reflect the fact that the period of calling activities does not strictly coincide with breeding periods, at least in typical prolonged breeders. The fact that \( P. \) gracilis males start to call earlier than the actual beginning of reproductive activities (i.e., when mature females appear) could overestimate the duration of the breeding season. As mentioned above, prior to the onset of breeding activities, calling may play a role in chorus formation rather than in attracting mates (Wiest 1982, Bastos & Haddad 1996). In these cases, calls by males in full reproductive potential may promote the endocrinial activation of other males to incite them into calling activity (Chu & Wilczynski 2001), thus increasing the chorus size as well as its attractiveness for females (Lucas & Howard 1995).

Regarding climatic variables, although reproductive activity patterns and temperature showed cyclical variation throughout the year, there was no significant correlation because most activity occurred in spring months and decreased during the warmer summer months. We also did not observe a correlation between reproductive activity and rainfall, which is probably due to the fact that rainfall was marked lower in the second year. Taken together these results suggest that neither temperature nor rainfall exclusively control the breeding patterns of \( P. \) gracilis in Uruguay. Moreover, the correlation of activity in both sexes with future changes in temperature suggests that internal mechanisms may have been involved in shaping similar activity patterns during the two consecutive seasons. The contribution of internal rhythms may be relevant considering their demonstrated role in alternatively inducing and inhibiting the gonadal activity of bufonid, ranid, and leptodactylid frogs (Jørgensen 1988, Mosconi et al. 1996, Tsai et al. 2003). Alternatively, the restriction of activity mainly to spring months may reflect an adaptation to avoid reproduction during summer months, when presumably higher evaporation occurs. As a pond dries up, increased crowding between amphibian larvae may result
in increased competition, growth inhibition, and cannibalism (Griffiths 1997). The study of the reproductive cycles of *P. gracilis* at lower and higher latitudes may provide insight into this apparent preference to reproduce at mild temperatures.

**Body size, fecundity and reproductive investments**

How to partition a finite amount of energy into maintenance, growth and reproduction is one of the major concerns of life history theory, and, thus, a very important issue in evolutionary biology. Life history theory predicts that reproduction costs in a given year has profound effects on subsequent performance, via changing reproduction and survival in subsequent years (Roff 1992, Stearns 1992). In ectothermic animals, growth rates decrease greatly after individuals reach sexual maturity (Halliday & Verrell 1988, Hemelaar 1988). Thus, for these species growth may be considered an investment in future reproduction since large females frequently produce more young than small females (Lardner & Loman 2003, and references therein). In addition, a reduction in growth due to reproduction costs could increase the probability of death by keeping the organism within a size range that is preferred by predators (Roff 1992).

The BSF relationship of *P. gracilis*, shows that a two slopes function explains much more of the total variance than a linear or an exponential model. One possible explanation for this is that pair-wise regression allows the separation of females that reproduce for the first time (and yet have great energy investment in growth) from those females that entered into the reproductive pool at least one year earlier. Reproductive investment data support this idea: of the 34 females in the low SVL group, 27 showed RI values lower than the mean, whereas of the 30 females in the high SVL group only 12 showed RI values lower than the mean. Thus, in small females advantages related to continued growth may impose an important limitation on the amount of energy that can be allocated to reproduction. In contrast, for larger females other more variable factors, such as food supply, competition or predation, may limit the amount of energy devoted to each reproductive event. In many amphibian species, fecundity is a plastic trait that can change with environmental conditions, among populations, among individuals within populations, and also among years within single individuals (Kaplan & King 1997, Morrison & Hero 2003, and references therein).

Finally, Bonnet *et al.* (2003) argued that studies based on hand-collected samples may overestimate the strength of the BSF relationship because low RI females are frequently discarded in the field as non-gravid females. It is possible that pitfall trapping outperforms the by-hand method in obtaining a representative sample of reproductively active *P. gracilis* females. However, pitfall traps may also undersample females with very high RI because they move less or stay hidden more often than other females (Bonnet *et al.* 2003, Shine 2003, and references therein). Thus, we suggest that a combination of manual collection and pitfall trapping may be more appropriate for assessing variation in the reproductive parameters of a given frog population.

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**References**


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