

Reproductive biology of an endemic *Physalaemus* of the Brazilian Atlantic forest, and the trade-off between clutch and egg size in terrestrial breeders of the *P. signifer* group

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Physalaemus crombiei is a small foam-nesting frog endemic to the Atlantic forest. It is a member of the *P. signifer* group known only from its type locality in Santa Teresa, State of Espírito Santo, and from another locality in the State of Bahia, Brazil. Most *Physalaemus* species are aquatic breeders, and species in the *P. signifer* group are the only ones exhibiting a tendency toward terrestrial reproduction in the genus. Here we describe the reproductive period, breeding site and reproductive modes of *P. crombiei* from a third population in the Atlantic forest, southeastern Brazil. We also investigated reproductive effort and size–fecundity relationships in females. Reproductive traits were compared to other species in the genus *Physalaemus*, especially those included in the *P. signifer* group. *Physalaemus crombiei* is a prolonged breeder, reproducing throughout the year with a peak of activity during the most rainy months (October–March). Males called from the humid forest floor and eggs embedded in foam nests were deposited in the water as well as on the humid floor amidst the leaf litter, or inside fallen leaves or tree holes containing rainwater on the forest floor. As expected, *P. crombiei* exhibited three alternative reproductive modes, as described for other species of the *P. signifer* group. The number of eggs produced per female varied from 91 to 250. Female body size is positively correlated both with ovary mass and clutch size (number of eggs per clutch). Variation in the number and size of eggs observed in *Physalaemus* species may be explained not only by female size, but also by the terrestrial reproductive mode exhibited by the species in the *P. signifer* group.

Key words: Amphibia, reproductive modes, reproductive effort, size–fecundity relationships

INTRODUCTION

Individual characteristics such as body size, age at first breeding, number of reproductive events and number and size of offspring are important components of the life history of organisms (Begon et al., 2006). Reproductive effort may be defined as the proportion of resources available to an organism of a given age that is allocated to reproduction during a defined period of time (Gadgil & Bossert, 1970). For females, reproductive investment can be viewed as a result of the combination of offspring number and the investment in each descendent (Stearns & Hoekstra, 2000). However, as the size of the body cavity and the capacity for resource acquisition are limited, there must be a compromise between egg number and size (Lüddecke, 2002). Since egg size and number affect survivorship and future parental investment with important consequences for fitness (Stearns, 1989), many studies have investigated the trade-off between number and size of eggs in different organisms and at different scales (e.g. Jetz et al., 2008; Wang et al., 2009). For anurans, reproductive output is influenced by female body size and condition (e.g. Castellano et al., 2004); egg size

and number also seem to be strongly influenced by the offspring's environmental conditions (e.g. Crump, 1981; Doughty, 2002; Dziminski & Roberts, 2006).

Anuran females are a suitable model to study patterns of reproductive allocation and reproductive trade-offs because, in most species, female reproductive effort is spent in egg production and parental care is absent, simplifying the quantification of reproductive parameters (Camargo et al., 2008). The ovarian complement of anurans with different reproductive modes is very diverse (Salthe & Duellman, 1973). Generally, large species produce more eggs than small species, and species that have generalized reproductive modes produce larger clutches than those with specialized modes (e.g. Crump, 1974; Prado & Haddad, 2005). In addition, within a given reproductive mode, there is a positive correlation between female body size and clutch size, and a negative correlation between clutch and egg size (Duellman, 1989). In anurans, this trade-off between clutch size and egg size is more pronounced in terrestrial breeders, which produce large eggs and small clutches (Salthe & Duellman, 1973). It has been suggested that small frogs would be more prone to experiment with terrestrial reproduction, since clutch

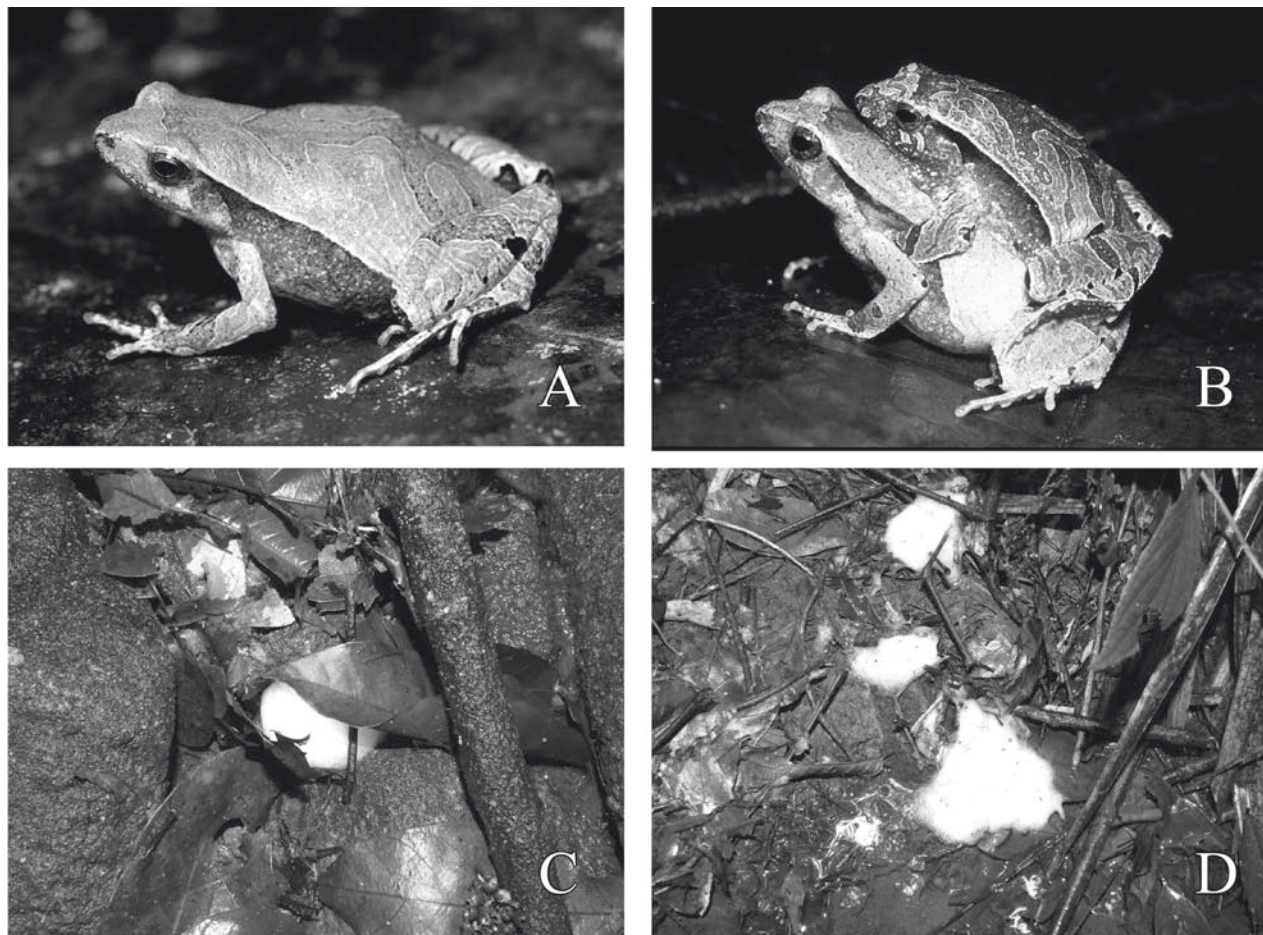


Fig. 1. Some aspects of the reproductive biology of *Physalaemus crombiei*: A) adult male; B) a pair in axillary amplexus; C) foam nest in the humid leaf litter; D) foam nests close to a rain puddle (Photos: A and B by J.L. Gasparini; C and D by B.B. Loureiro).

size is already small, and hence selection would favour a minor decrease in egg number and an increase in egg and hatchling size (Salthe & Duellman, 1973).

Frogs of the genus *Physalaemus* generally deposit a large number of eggs in foam nests and tadpoles develop in puddles (e. g. Lynch, 1971). Foam nests are considered an adaptation to environments with unpredictable rains, as the foam provides protection for eggs and tadpoles against desiccation (Heyer, 1969; Duellman & Trueb, 1986) as well as predators (Downie, 1990; Menin & Giaretta, 2003, Giaretta & Menin, 2004). The genus *Physalaemus* contains species that occur in a wide variety of habitats, from the leaf litter of rainforests (Heyer et al., 1990) to seasonal habitats such as the Brazilian savanna (cerrado), chaco and caatinga (Cei, 1980; Frost, 2009). Despite being composed of relatively common species of broad geographical distribution (Frost, 2009), few studies of their ecology and behaviour are available (e.g. Giaretta & Menin, 2004; Brasileiro & Martins, 2006).

Physalaemus crombiei Heyer & Wolf, 1989 (Fig. 1) is an endemic species of the Atlantic forest known from two localities: the type locality in Espírito Santo state, and another in the state of Bahia (Frost, 2009). Except for some information provided in the species' description (Heyer & Wolf, 1989), no information on the biology of *P. crombiei*

is available. This species belongs to the *P. signifer* group, which is composed of 13 species endemic to the Atlantic forest (Nascimento et al., 2005; Cruz et al., 2007): *P. angrensis* Weber, Gonzaga & Carvalho e Silva, 2006, *P. atlanticus* Haddad & Sazima, 2004, *P. bokermanni* Cardoso & Haddad, 1985, *P. caete* Pombal & Madureira, 1997, *P. camacan* Pimenta, Cruz & Silvano, 2005, *P. crombiei* Heyer & Wolf, 1989, *P. irroratus* Cruz, Nascimento & Feio, 2007, *P. maculiventris* (Lutz, 1925), *P. moreirae* (Miranda-Ribeiro, 1937), *P. nanus* (Boulenger, 1888), *P. obtectus* Bokermann, 1966, *P. signifer* (Girard, 1853) and *P. spiniger* (Miranda-Ribeiro, 1926). Within this species group, three alternative reproductive modes have been described: 1) foam nest deposited in puddles and exotrophic tadpoles in puddles (aquatic typical mode for the genus *Physalaemus*); 2) foam nest on the humid forest floor close to puddles and tadpoles in puddles; and 3) foam nest in water accumulated in axils of terrestrial bromeliads and tadpoles in puddles (Haddad & Pombal, 1998; Haddad & Prado, 2005). While most *Physalaemus* species are aquatic breeders, species in the *P. signifer* group exhibit a tendency toward terrestrial reproduction.

In the present study, we report on the reproductive biology of *P. crombiei* from a third known population in the Atlantic forest of Espírito Santo state, Brazil. Our aims

Table 1. Snout–vent length (SVL), number of mature oocytes/eggs, mature oocyte/egg diameter, reproductive modes and sexual size dimorphism (SSD) for *P. crombiei* (this study) and other 12 *Physalaemus* species. Reproductive modes: 11) foam nest floating on pond, exotrophic tadpoles in ponds; 14) foam nest on water accumulated in the axils of terrestrial bromeliads and its variations (see text), exotrophic tadpoles in ponds; 28) foam nest on the humid forest floor, tadpoles in ponds (Haddad & Pombal, 1998; Haddad & Prado, 2005). Sexual size dimorphism (SSD): Y = yes; N = no. (*) Species in the *Physalaemus signifer* group (*sensu* Nascimento et al., 2005).

Species	Female SVL (mm)	Number of eggs/oocytes	Egg/oocyte diameter (mm)	Reproductive mode	SSD
<i>P. albonotatus</i>	23.5 ^m	719 ^m	1 ^m	11 ^m	Y ^k
<i>P. atlanticus</i> *	22.4 ^h	81 ^h	1.6 ^h	11 and 28 ^h	Y ^k
<i>P. biligonigerus</i>	30 ^b	1020 ^b	–	11 ^b	Y ^k
<i>P. centralis</i>	34.5 ^a	1872 ^a	1.3 ^a	11 ^a	N ^k
<i>P. crombiei</i> *	22.43	158	1.38	11, 14, and 28	Y ^k
<i>P. cuvieri</i>	28.96 ⁿ	584.46 ⁿ	1.87 ⁿ	11 ⁿ	Y ^k
<i>P. ephippifer</i>	31 ^j	410 ^j	–	11 ^j	–
<i>P. gracilis</i>	29.9 ^b	311 ^b	–	11 ^{q,r}	Y ^k
<i>P. henselii</i>	23.1 ^p	296.9 ^p	0.9 ^q	11 ^q	–
<i>P. cf. marmoratus</i>	39.3 ^e	2465 ^e	1.4 ^e	11 ^{c,e}	Y ^k
<i>P. olfersii</i>	31.4 ⁱ	959 ⁱ	1.6 ⁱ	11 ^{d,i}	N ^k
<i>P. signifer</i> *	27.4 ^o	273 ^o	–	11 and 28 ^o	Y ^k
<i>P. spiniger</i> *	21.1 ^f	29.7 ^f	1.62 ^f	11, 14, and 28 ^f	Y ^k

Sources: a) Brasileiro & Martins (2006); b) Camargo et al. (2008); c) C.P.A. Prado (pers. obs.); d) C.F.B. Haddad (pers. obs.); e) Giaretta & Menin (2004); f) Haddad & Pombal (1998); g) Haddad & Prado (2005); h) Haddad & Sazima (2004); i) Heyer et al. (1990); j) Hödl (1990); k) Nascimento et al. (2005); l) Pombal Jr. (1995); m) Prado & Haddad (2005); n) N.C. Pupin (unpubl. data); o) Wogel et al. (2002); p) Maneyro et al. (2008); q) Barrio (1965); r) Camargo et al. (2005).

were to 1) describe the breeding activity period, breeding habitat and reproductive mode of the species; 2) determine clutch size and egg size, estimate reproductive effort and investigate female size–fecundity relationships; and 3) test the prediction that clutch size should be smaller while egg size should be larger in the terrestrial breeders of the *P. signifer* group compared to other *Physalaemus* species with obligatory aquatic reproduction (Haddad & Prado, 2005), following the general pattern observed in anurans (Salthe & Duellman, 1973).

MATERIALS AND METHODS

Study site

The study was carried out in the municipality of Aracruz, Espírito Santo state, southeastern Brazil, in a remnant of Atlantic forest (19°49'12"S; 40°16'22"W) surrounded by eucalyptus plantations. Collections were conducted near a temporary creek within the native forest. The climate is hot and humid throughout the year with rains concentrated from October to February. In the study year, the average temperature was 23.9 °C and the total annual rainfall was 1401 mm. The original vegetation cover is a lowland Atlantic forest known as *tabuleiro* forest that, together with the other types of Atlantic forest and with the *restinga* (sand dune), compose the ecosystems of the

Brazilian coast (see Villela et al., 2006). The study area is located approximately 53 km from the type locality of *P. crombiei*, the Biological Reserve of Nova Lombardia, Santa Teresa, Espírito Santo state, southeastern Brazil.

Data collection and analysis

Data were collected monthly from November 1995 to November 1996. The reproductive period was determined based on direct observations of calling males, and presence of gravid females or foam nests. The reproductive habitat was described based on the vegetation cover and type of substrate that males called from, and the type of water body or substrate used for egg deposition.

Specimens were collected using pitfall traps with drift fences, prepared and fixed as described by McDiarmid (1994) and deposited in the Collection of Amphibians CFBH, Departamento de Zoologia, I.B., Universidade Estadual Paulista (UNESP), Rio Claro, São Paulo State, Brazil. The snout–vent length (SVL) of adults was measured with calipers to the nearest 0.1 mm, and the body and gonad masses of females were measured in a digital scale to the nearest 0.001 g. The diameters of 10 mature oocytes from each female were measured to the nearest 0.1 mm with an ocular micrometer under a stereomicroscope. The average number of eggs per clutch was estimated based on the number of mature oocytes in the females' ovaries.

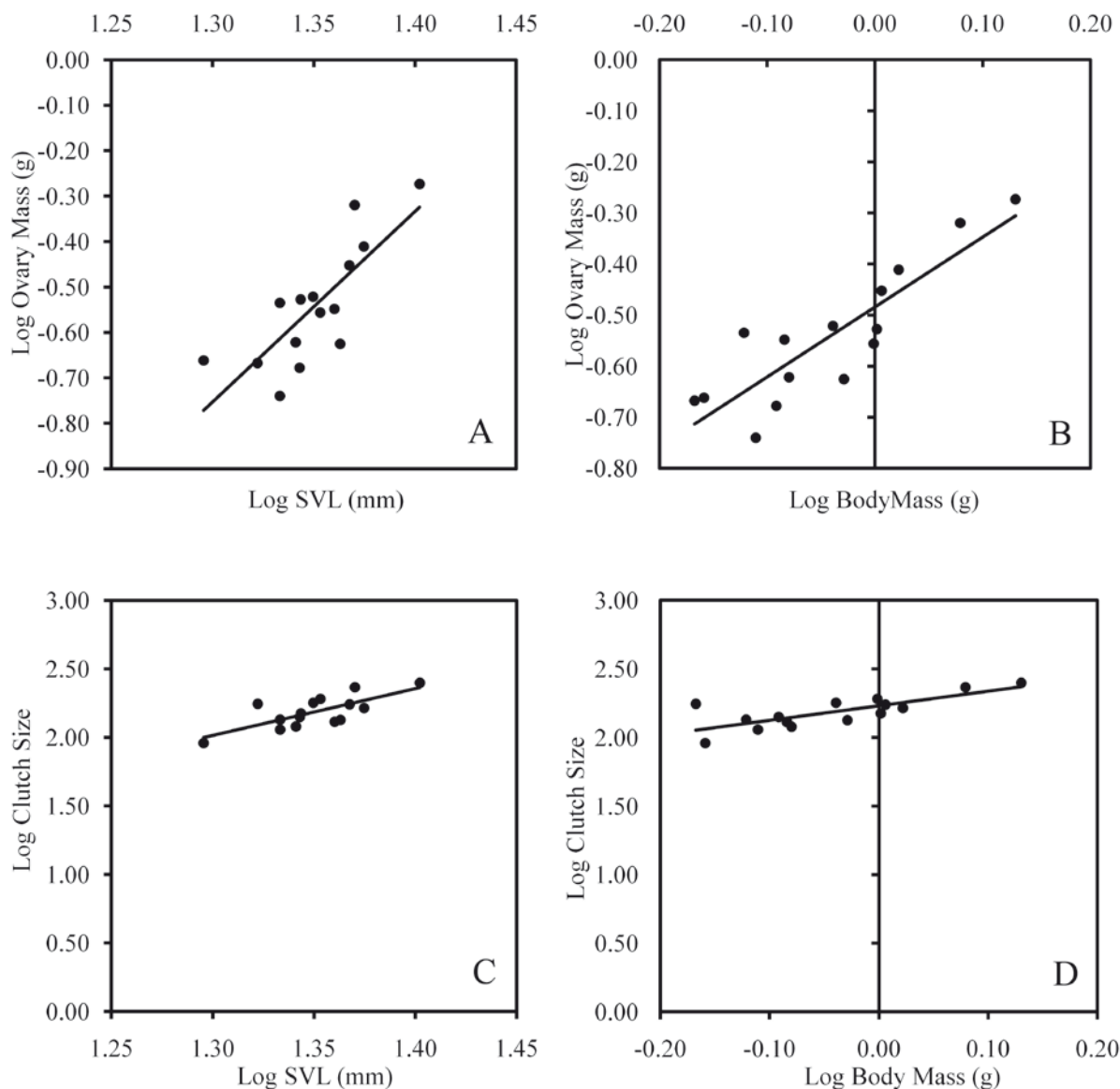


Fig. 2. Size–fecundity relationships in 15 *P. crombiei* females from Aracruz, Espírito Santo state: A) log snout–vent length (SVL) versus log ovary mass ($y = -6.19 + 4.18x$); B) log body mass vs log ovary mass ($y = -0.48 + 1.36x$); C) log SVL vs log clutch size ($y = -2.44 + 3.42x$); D) log body mass vs log clutch size ($y = 2.23 + 1.06x$).

Although far from ideal, the most common way to measure reproductive effort is to establish the ratio between gonad mass and body mass or clutch volume and body volume (e.g. Prado & Haddad, 2005; Rodrigues et al., 2005). Thus, the reproductive effort of *P. crombiei* females was estimated using the percentage of ovary mass relative to body mass.

Relationships between female fecundity and female body size were examined through simple linear regression analysis (Zar, 1999) with the following variables: 1) snout–vent length (SVL) and clutch size, i. e. number of mature oocytes/eggs per clutch; 2) body mass and clutch size; 3) SVL and ovary mass; 4) body mass and ovary mass. Although gravid females were found in almost all months studied, only those captured in November 1995 were included in the analyses, to avoid differences in

clutch and egg size caused by differences in environmental conditions (e.g. water and food availability). Student's *t* test was used to compare SVL and body mass of adult males and females (Zar, 1999).

Because eggs tend to be larger while clutch size tends to be smaller in anurans with terrestrial reproductive modes (e.g. Salthe & Duellmann, 1973; Crump, 1974), we investigated egg size and clutch size in the *P. signifer* group, which exhibit more terrestrial modes, in comparison to those of other species in the genus. To minimize the effect of body size, the egg diameter/female SVL ratio was used in the subsequent analyses. Data on SVL, clutch size (number of oocytes/eggs per clutch), egg diameter (ED), reproductive mode, reproductive patterns and sexual dimorphism of 12 other *Physalaemus* species were obtained from the literature (Table 1). Our data on *P. crombiei* were

Table 2. Comparisons of mean snout–vent length (SVL) and mean mass of male and female *P. crombiei* from the municipality of Aracruz, Espírito Santo state, southeastern Brazil.

	Males			Females			<i>t</i>	<i>P</i>
	<i>n</i>	Mean ± SD	Range	<i>n</i>	Mean ± SD	Range		
SVL (mm)	15	19.23±0.88	18.65–21.71	15	22.43±1.29	19.75–25.25	1.7	<0.001
Mass (g)	15	0.68±0.06	0.6–0.8	15	0.92±0.18	0.68–1.35	1.7	<0.001

compared to those of the 12 *Physalaemus* species, except for egg size, values for which were found in the literature for eight species only. Linear regressions (Zar, 1999) were calculated between the following variables for the *Physalaemus* species: 1) SVL and clutch size, 2) SVL and ED, 3) clutch size and ED, 4) SVL and ED/SVL and 5) clutch size and ED/SVL. The non-parametric Mann–Whitney test was used to compare egg size and clutch size of the species in the *Physalaemus signifer* group with those of other species in the genus.

RESULTS

Breeding activity pattern and reproductive modes

Calling males, gravid females and foam nests of *P. crombiei* were observed throughout the year, with a peak of activity in the wettest months (October to March). Individuals were observed at the edge of and in the forest, in eucalyptus plantations with understory, and at the margins of creeks within the forest. Males called after rains and remained calling while accumulated water was available. Frogs used the moist forest litter as calling sites, hidden beneath branches and leaves or exposed on the ground.

Three alternative sites were used by the species to deposit foam nests. Clutches were observed in small puddles that formed on the roads after heavy rains or inside fallen leaves or tree holes on the forest floor containing rainwater, but also on the humid floor amidst the leaf litter with no water (Fig. 1). The average number of eggs per clutch (estimated from mature oocytes) was 158.73±43.25 (range 91–250; *n*=15 females) and the average ovary mass was 0.30±0.1 g (range 0.18–0.53; *n*=15). Mature eggs are unpigmented with an average diameter of 1.38±0.064 mm (range 1.25–1.49; *n*=150 eggs from 15 females). All ovaries examined contained only mature oocytes.

Size–fecundity relationships and reproductive effort

The average reproductive effort (ovary mass/body mass) was 32.06% ± 5.26 (range 23.48–39.91; *n*=15). For females, a positive correlation was found between SVL and ovary mass ($r^2=0.62$; $P<0.01$; *n*=15) (Fig. 2a), body mass and ovary mass ($r^2=0.75$; $P<0.01$; *n*=15) (Fig. 2b), SVL and clutch size ($r^2=0.54$; $P<0.01$; *n*=15) (Fig. 2c), as well as body mass and clutch size ($r^2=0.59$; $P<0.01$; *n*=15) (Fig. 2d). Female *P. crombiei* were larger ($t=1.7$; $P<0.001$; *n*=15) and heavier than males ($t=1.7$; $P<0.001$; *n*=15, Table 2).

Reproductive traits compared among *Physalaemus* species are shown in Table 1. A significant positive correlation was found between SVL and clutch size ($r^2=0.69$; $P<0.01$; *n*=13, Fig. 3a), but not between SVL and ED ($r^2=0.003$; $P=0.87$; *n*=9) or between clutch size and ED ($r^2=0.0005$; $P=0.95$; *n*=9, Figs 3b and 3c, respectively). Significant negative correlations were observed between SVL and ED/SVL ($r^2=0.44$; $P<0.05$; *n*=9, Fig. 3d) and between clutch size and ED/SVL ($r^2=0.46$; $P<0.05$; *n*=9, Fig. 3e). The mean number of eggs per clutch was smaller in species of the *P. signifer* group (mean = 143±103; range 29.7–273; *n*=4) compared to those of other species of the genus (mean = 959.7±746.9; range 296.9–2465; *n*=9; Mann–Whitney $Z(U) = 2.77$; $P<0.01$). Relative egg size (ED/SVL ratio) was significantly larger in species of the *P. signifer* group (mean = 0.07±0.007; range 0.062–0.077; *n*=3) than in other *Physalaemus* species (mean = 0.04±0.004; range 0.036–0.048; *n*=6; Mann–Whitney $Z(U) = 2.32$; $P=0.01$).

DISCUSSION

Breeding activity pattern and reproductive modes

Physalaemus crombiei differs from most *Physalaemus* species in being able to reproduce throughout the year, with a peak of activity in the rainy season. Many species of this genus have long breeding seasons (Cardoso, 1981; Haddad & Pombal, 1998; Wogel et al., 2002; Brasileiro & Martins, 2006), but most of them only reproduce during a few months in the rainy season rather than throughout the entire year (Rodrigues et al., 2004; Camargo et al., 2005; Brasileiro & Martins, 2006). The continuous breeding pattern of *P. crombiei* might be considered uncommon, since even species such as *P. spiniger* (Haddad & Pombal, 1998) and *P. atlanticus* (Haddad & Sazima, 2004), which also inhabit the Atlantic forest, were not observed breeding beyond the rainy season. Although breeding events for *P. crombiei* were recorded throughout the year, the peak in breeding activity occurred during the rainy season, possibly due to greater availability and stability of puddles on the forest floor.

Physalaemus crombiei exhibited three alternative reproductive modes, as described for *P. spiniger* (Haddad & Pombal, 1998; see Table 1). In addition to the typical reproductive mode of the genus *Physalaemus* (Haddad & Prado, 2005), in which foam nests are deposited on the water in puddles (mode 11), clutches were also deposited on moist forest litter (mode 28), as described for

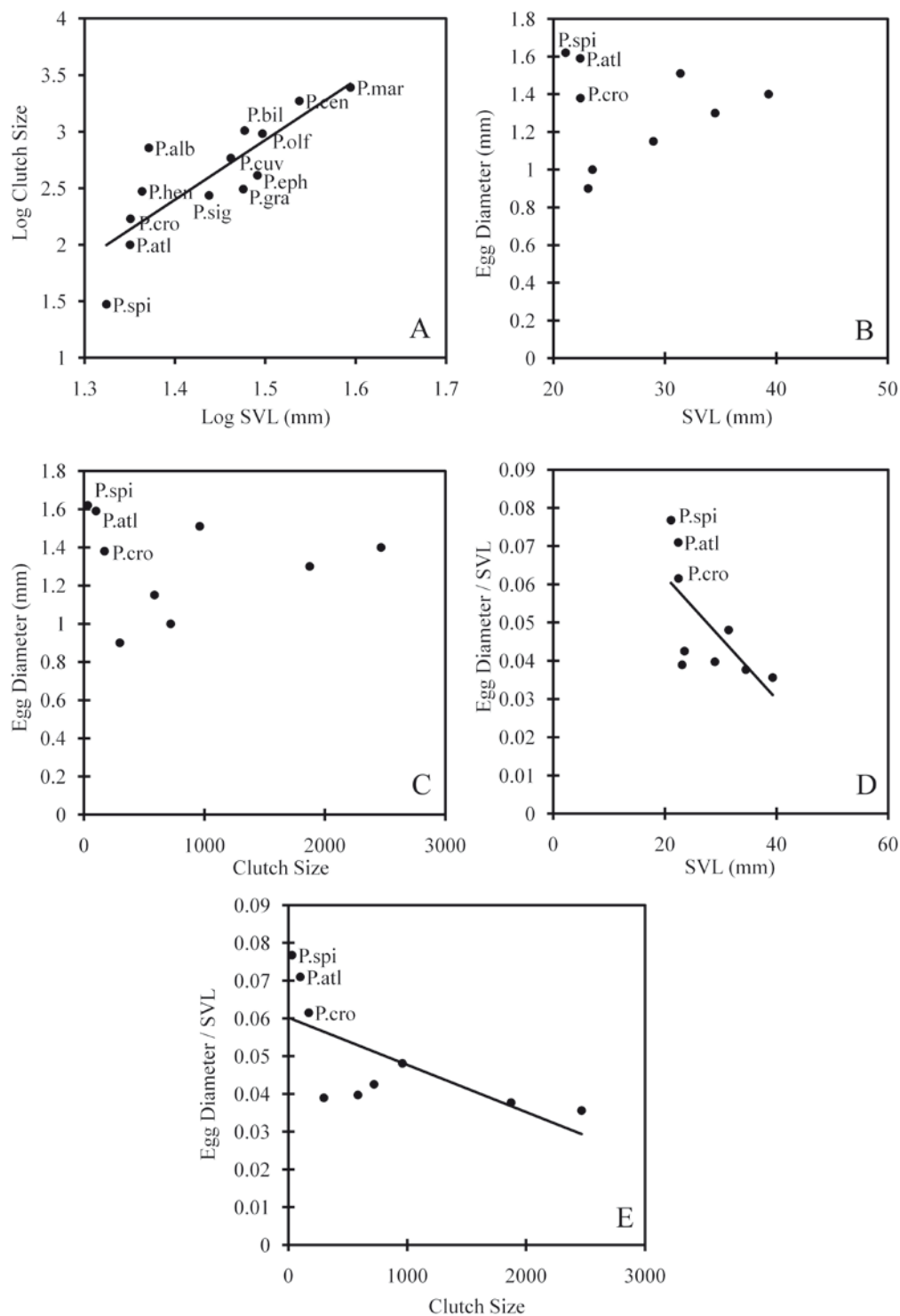


Fig. 3. Correlations between snout-vent length (SVL), clutch size and egg/oocyte diameter in 13 species in the genus *Physalaemus*: A) log SVL vs clutch size ($y = -4.9 + 5.2x$); B) SVL vs egg diameter; C) clutch size vs egg diameter; D) SVL vs egg diameter/SVL ($y = 0.009 - 0.001x$); and E) clutch size vs egg diameter/SVL ($y = 0.06 - 1.2^{-5} x$). Species: *P. albonotatus* (P.alb), *P. atlanticus* (P.atl), *P. biligonigerus* (P.bil), *P. centralis* (P.cen), *P. crombiei* (P.cro), *P. cuvieri* (P.cuv), *P. ephippifer* (P.eph), *P. gracilis* (P.gra), *P. henselli* (P.hen), *P. cf. marmoratus* (P.mar), *P. olfersii* (P.olf), *P. signifer* (P.sig), *P. spiniger* (P.spi).

P. atlanticus (Haddad & Sazima, 2004), *P. spiniger*, *P. bokermanni*, and *P. signifer* (Haddad & Pombal, 1998; Wogel et al., 2002). A third mode, in which foam nests were observed inside fallen leaves or in tree holes on the forest floor containing rainwater, is here interpreted as

variations of mode 14, described as foam nests in water accumulated in axils of terrestrial bromeliads and tadpoles in puddles for *P. spiniger* (Haddad & Pombal, 1998). Variations of mode 14 were also observed for *P. caete* (foam nests inside tree holes with water near the

ground; Pombal & Madureira, 1997) and *P. erythros* (foam nests within flooded hole in the ground or flooded rock crevices; Baêta et al., 2007). All species mentioned above are included in the *P. signifer* group, except for *P. erythros* which belongs to the *P. deimaticus* group (*sensu* Nascimento et al., 2005). The embryos and/or tadpoles from foam nests of *P. crombiei* deposited on the moist litter are probably washed into temporary puddles after rains, where tadpoles feed and complete their development (Haddad & Pombal, 1998; Haddad & Prado, 2005). As observed for *P. spiniger* (Haddad & Pombal, 1998), clutches of *P. crombiei* deposited on moist litter with no water accumulated nearby might be an alternative, apparently associated with high air humidity and the almost constant soil moisture of the Atlantic forest. In this case, the survival of the offspring is facilitated by the presence of the foam nest surrounding the eggs and larvae, as the foam prevents desiccation (Heyer, 1969), allowing initial development to occur in the absence of water. This strategy may provide a competitive advantage over other anuran species, as *P. crombiei* tadpoles may be the first to occupy water puddles after rains. Also, tadpoles of *P. crombiei* may benefit from fewer predators (Magnusson & Hero, 1991), which is correlated to the existence time of a puddle (Giaretta & Menin, 2004).

Size–fecundity relationships and reproductive effort in *P. crombiei*

The average number of eggs produced by *P. crombiei* females was lower than those of most other *Physalaemus* species (see Table 1 for species lists and references), which is expected given the smaller SVL and the positive correlation between clutch size and female body size commonly reported for anurans (Crump, 1974; Halliday & Verrell, 1986; Prado & Haddad, 2005). Nonetheless, *P. crombiei* produced more eggs than *P. atlanticus* (Haddad & Sazima, 2004), despite similar body sizes. The number of eggs produced by a female might differ depending on the reproductive mode of the species, as the number of eggs tends to decrease and the size of eggs tends to increase from aquatic to more terrestrial modes (Salthe & Duellman, 1973; Crump, 1974; Duellman & Trueb, 1986). *Physalaemus crombiei* and *P. atlanticus* produce few, large eggs, have similar SVLs, and identical reproductive modes including foam nests deposited on the forest floor. The more terrestrial reproductive mode would explain the low fecundity, but not the differences in egg number, which may be caused by different durations of the reproductive period and the number of clutches females can produce in a single season (e.g. Prado & Haddad, 2005), or habitat differences related to climatic conditions and consequently the amount of available resources (e.g. Pradario & Robinson, 1990; Lüddecke, 2002).

Although *P. crombiei* females produced lower numbers of eggs compared to most *Physalaemus* species, the reproductive effort was high, as ovary mass accounted for approximately 32% of body mass. We must consider the possibility that the reproductive output of *P. crombiei* females might have been underestimated, since there is no information on how many clutches a female is able to deposit during a year. Few studies have reported on

reproductive investment in females of *Physalaemus* species; *P. albonotatus* females invested from 16.6% (Prado & Haddad, 2005) to 27% (Rodrigues et al., 2004) in ovary mass in two different regions in central Brazil. For another foam-nesting leiuperid frog from a different genus, *Eupemphix nattereri*, females were reported to invest around 22% in ovary mass (Rodrigues et al., 2004). In a study conducted in the Pantanal floodplain in central Brazil, 11 species from different families invested between 5.5% and 18% of their body mass in ovaries (Prado & Haddad, 2005). Thus, the investment by *P. crombiei* can be seen as high, and might be related to species' ecological traits and/or environmental conditions such as number of reproductive events, predation pressure on eggs and/or tadpoles, reproductive sites and prey availability. Studies addressing these questions need to be conducted to elucidate such variation in reproductive effort.

The number of eggs was positively correlated with SVL and female mass, as reported in previous studies on other anuran species (Rodrigues et al., 2004; Camargo et al., 2005; Prado & Haddad, 2005). However, a stronger relationship was found between the number of eggs and body mass than SVL, and an even stronger relationship between body mass and ovary mass. This demonstrates that body mass is the most appropriate variable for estimating the reproductive potential of females, representing the size of the female body cavity as well as its nutritional condition (Prado & Haddad, 2005).

Physalaemus crombiei exhibits sexual dimorphism, with females being on average larger than males. This is a common pattern for anurans (Shine, 1979), including most species of the genus *Physalaemus* (Table 1). Several factors might be responsible for this pattern, such as pressure for female size increase due to increase in fecundity (Crump & Kaplan, 1979), earlier sexual maturation of males, differential predation on larger males (Howard, 1981), or even restrictions on male growth due to energy expenditure during reproductive activities (Woolbright, 1989). Intra- or intersexual selection can also lead to differences in size between males and females (e.g. Ryan & Keddy-Hector, 1992; Brasileiro & Martins, 2006). For the genus *Physalaemus*, the different selective pressures leading to sexual size dimorphism still need to be discerned from each other.

The trade-off between number and size of eggs in terrestrial *Physalaemus* breeders

Although the number of eggs produced by females of the genus *Physalaemus* was correlated with female size, the relationships between SVL and egg diameter (ED), and between clutch size (number of mature eggs) and ED, were not significant. Despite our small sample size, the eggs of species of the *P. signifer* group were significantly larger compared to those of other species. However, correlations of the same variables (SVL and clutch size) with the ED/SVL ratio proved to be significantly negative, suggesting that body size might have an effect. Eggs of species that deposit terrestrial foam nests (*P. signifer* group: *P. atlanticus*, *P. crombiei* and *P. spiniger*) are larger but fewer in number than those of species that lay foam nests on water. Thus, the observed trade-off between number and size of

eggs might be due to a tendency towards terrestriality in the species of the *P. signifer* group, corroborating our initial prediction. More terrestrial modes apparently require more yolk for development, maintaining the embryos until the clutch is carried to temporary puddles where tadpoles will feed and complete their development (Salthe & Duellman, 1973). Furthermore, larger eggs result in larger size at metamorphosis, which may be advantageous for offspring and parental fitness since the early stages of anuran lives are more vulnerable to predation (Magnusson & Hero, 1991; Prado et al., 2005; Dziminski et al., 2009).

Salthe & Duellman (1973) hypothesized that selection would favour increased clutch size in larger frogs and increased egg size in smaller frogs. Since clutch size is already small in the latter species, selection would favour a small decrease in egg number, increasing egg size and consequently hatchling size. Moreover, these authors suggested that only small to medium sized frogs are able to engage in terrestrial reproductive modes. Comparing species in the genus *Physalaemus*, such predictions seem plausible. *Physalaemus* species in the *signifer* group are smaller compared to most species in the genus. Other small *Physalaemus* species, such as *P. albonotatus*, also exhibit the typical aquatic mode (Rodrigues et al., 2004), but inhabit seasonal areas with a pronounced dry season, precluding the evolution of more terrestrial modes due to egg desiccation risks (e.g. Prado & Haddad, 2005; Brasileiro & Martins, 2006), whereas species in the *P. signifer* group are inhabitants of the Atlantic forest with continuously high air humidity and rainfall. This general trend can also be observed across 39 reproductive modes described for frogs mainly from the Atlantic forest, where terrestrial modes mostly involve small to medium sized species such as members of the genera *Brachycephalus*, *Flectonotus*, *Paratelmatobius* and *Zachaeus* (Haddad & Prado, 2005).

Given the diverse reproductive strategies exhibited by tropical anurans, our results reinforce the need for more ecological and behavioural studies allied with comparative phylogenetic methods. Such studies can help to elucidate the evolution of egg size and number trade-off as well as reproductive modes in frogs. Furthermore, given the extinction threat many anuran species have been experiencing all over the world (e.g. Becker et al., 2007), integrating information on species life-history traits with those of landscape configuration may also be useful in the identification of priority areas for conservation, producing more effective conservation strategies (e.g. Loyola et al., 2008; Becker et al., 2009).

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