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Seasonal variation in body size of tropical anuran amphibians

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Abstract. Most anurans are considered short lived reproducing only once, with adult size being an important factor contributing to mating success. We sampled anurans with 30 pitfall traps in three forest patches of the northwestern São Paulo State, Brazil, throughout one year. Our objective was to analyze the variation in anuran body size between the dry and rainy season. Our hypothesis is that anurans registered during the rainy season will be larger than conspecifics registered during dry season, since there is a selective advantage in having a larger body size during reproduction activity. The most abundant species registered in the patches were *Eupemphix nattereri*, *Physalaemus cuvieri* and *Leptodactylus podicipinus*. Males and females of *E. nattereri* and *P. cuvieri* recorded in the rainy season were larger than of those captured in the dry one. In contrast, males of *L. podicipinus* were larger in the dry season than in the rainy season. Besides, the operational sex ratio (OSR) in the breeding season was biased towards males in these three species with, respectively, 4.66, 3.2 and 1.87 males per female. Our results suggest that probably body size variation between different seasons is a consequence of individuals' turnover between rainy seasons. Lower individuals captured in the dry season, would be reproductively active adults in the next breeding season.

Keywords. Mesophytic semideciduous forest; seasonality, recruitment, success of reproduction.

Introduction

Many studies on temperate and tropical anuran amphibians show that the size of males during the reproductive season is important, because large males have more access to females because they are better fighters or are more attractive to females (Wilbur, Rubenstein and Fairchild, 1978; Ryan, 1980; Berven, 1981; Duellman and Trueb, 1986); and larger females produce larger eggs or spawning in comparison to smaller conspecific females (Crump, 1974; Woolbright, 1983; Lang, 1995; Halliday and Tejedo, 1995; Hettyey, Torok and Hévizi, 2005) and have a greater probability of producing more than one clutch per reproductive season (Howard, 1978; Telford and Dyson 1990). According to Kupfer (2007), the typical female-biased sexual size dimorphism in amphibians may be partly explained by sex-specific growth trajectories and delayed maturity of females, while male-bias is associated with sexual selection for large males through territoriality and male-male combat. However, the effect of varying adult size on fitness of Neotropical frogs has been little studied (Wogel, Abrunhosa and Pombal, 2002; Wogel and Pombal, 2007).

Most tropical anurans restrict their reproductive activity to rainy periods (e.g., Rossa-Feres and Jim, 1994; Toledo, Zina and Haddad, 2003; Brasileiro et al. 2005; Vasconcelos and Rossa-Feres, 2005; Santos, Rossa-Feres and Casatti, 2007). For a high reproductive success during the reproductive season individuals need to remain at the breeding site for a longer time period trying to attract females by calling or actively searching for a mate. Consequently, this period should also provide a greater risk that these individuals are predated by both auditory exposure (male vocal) and the displacement in the search for partners (Trivers, 1972; Iwasa and Odendaal, 1984).

Most anurans are considered short lived and likely only breed once (Richter and Seigel, 2002; Giaretta and Menin, 2004; Werner et al., 2007). According to Donnely (1999), the diminishing of the size of adult frogs over time may indicate events of recruitment. Here, our objective was to verify if anuran body size varies among conspecifics registered in the dry and

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Figure 1. Mean ± SE of snout-vent length (SVL) of females and males of *Eupemphix nattereri* captured in the rainy season (dark bar) and dry season (light bar). Same letters indicate difference between rainy and dry season ($p < 0.05$). The numbers above the bars indicate sample sizes.

in the rainy season. Our hypothesis is that anurans registered during the rainy season will be larger than conspecifics registered during the dry season, since there is a selective advantage in having a larger body size during reproduction activity (Crump, 1974; Wilbur, Rubenstein and Fairchild, 1978; Ryan, 1980; Berven, 1981; Woolbright, 1983; Lang, 1995; Halliday and Tejedo, 1995). Moreover, others studies show, the operational sex ratio is biased towards males during reproduction activity in temperate zones, but little is known about operational sex ratio for Neotropical anurans. Therefore, with a view to supplying information on the seasonal variation of adult size and sex ratio in anurans, we sought to answer the following questions: 1) is there any difference in body size of conspecifics (males and females) anurans in relation to the rainy and dry periods? and 2) what is the operational sex ratio of males to females during reproduction activity?

Material and Methods

This study was carried out in three forest fragments, distant 1 to 4 km from each other in the Icém Municipality (20º22' S, 49º19' W), Northwestern São Paulo State, Brazil. The anuran fauna was sampled throughout November 2004 to July 2005, using pitfall traps (n = 10 in each remnant), installed along two 20 m long transects, parallel at 10 m apart from each other, with each transect containing five buried plastic buckets (3 l) equidistant 5 m and without drift fences (see Silva and Rossa-Feres, 2007).

The three patches analyzed have breeding areas next to the border (maximum distance 50 m) where these species were registered during the calling period. According to Silva and Rossa-Feres (2007), forest remnants in this region are used by anurans as shelter and/or for displacement between breeding sites. Therefore, we suggest that the individuals caught in the pitfall traps installed in the forest remnants were displacing to or from breeding sites.

The original vegetation of this region is Mesophytic Semideciduous Forest, nowadays reduced to small remnants embedded in a matrix of diverse cultures, mostly cattle pasture and sugar cane (Rodrigues et al., 2008). The climate is hot and humid tropical, Aw of Köppen (Peel, Finlayson and McMahon, 2007), with two well defined climatic seasons: rainy, between October and March, and a pronounced dry season, between April and September, which receives only 15 % of the annual total precipitation which varies between 1100 and 1250 mm (± 225 mm) (Barcha and Arid, 1971; Vasconcelos and Rossa-Feres, 2005; Santos, Rossa-Feres and Casatti, 2007).

To investigate the variation in the snout-vent length (SVL) of males and females between the breeding and dry seasons, the study period was divided according to the rain regime: i) rainy season (November to February, with 811.6 mm of precipitation), and ii) dry season (March to July, with 357 mm). The anurans captured in the pitfall traps were sexed by the examination of their external morphology (presence of vocal sac and nuptial spine in males) and of their gonads. The snout-vent length (SVL) of the individuals was measured with 0.1 mm precision calipers. The differences in the SVL between the seasons were compared by Student's t test (Zar, 1999). These analyses were performed using the StatSoft (2004) computer program. The operational sex ratio (OSR), defined as the average ratio of sexually active males to fertilizable females (Emlen, 1976), was calculated dividing the number of males by the number of females captured only in the rainy season, period when species are in reproductive activity (Vasconcelos and Rossa-Feres, 2005; Santos, Rossa-Feres and Casatti, 2007). Specimens captured were fixed in 10 % formalin and stored in 70 % ethyl alcohol solution. Voucher specimens were deposited in the scientific collection of amphibians of the Department of Zoology and Botany of the UNESP, Campus of São José de Rio Preto, São Paulo, Brazil (DZSJRP: *E. nattereri* - 10099-10115, 10169-10192, 10224-10234, 10263-10272; *P. cuvieri* – 10094-10097, 10116-10122, 10137, 10145, 10149-10153, 10155-10157, 10177-10178, 10205, 10207-10210, 10217-10223; *L. podicipinus* – 10124-10126, 10133, 10138, 10158, 10161-10168, 10174-10176, 10211-10216, 10237-10239, 10262, 10279, 10283-10288).

Results

The most abundant amphibians within the forest patches were: *Eupemphix nattereri* Steindachner 1863 (74 individuals), *Physalaemus cuvieri* Fitzinger 1826 (35 individuals) and *Leptodactylus podicipinus* (Cope 1862) (46 individuals). The full list of species occurring in the study area can be found in Silva and Rossa-Feres (2007). Individuals of *E. nattereri* captured in the rainy season were larger [X ± SD SVL: males = 3.69 ± 0.42 (n = 28); females = 4.57 ± 0.40 (n = 6)] than those captured in the dry season (X ± SD SVL: males = 3.26 ± 0.30 (n = 23); females = 3.46 ± 0.46 (n = 17); Student's t test - males: $t = 4.15$; $p < 0.001$; females: $t = 5.20$; $p <$

0.001; Figure 1). Similar results was found for *P. cuvieri* with larger individuals captured in the rainy season [X ± SD SVL: males = 2.84 ± 0.18 (n = 15); females = 3.07 ± 0.21 (n = 15)] than in the dry season (X ± SD SVL: males = 2.42 ± 0.23 (n = 3); females = 2.35 ± 0.24 (n = 9); Student's t test - males: $t = 3.56$; $p < 0.001$; female: $t = 6.56$; $p < 0.001$; Figure 2). Differently, males of *L. podicipinus* were larger in the dry season [X ± SD SVL: males = 3.10 ± 0.37 (n = 18); females = 3.45 ± 0.39 (n = 7)] than in the rainy season (X ± SD SVL: males = 2.77 ± 0.36 (n = 16); females = 2.97 ± 0.52 (n = 5); Student's t test - males: t =2.60; $p < 0.01$; females t = 1.82; $p = 0.09$; Figure 3). *Eupemphix nattereri* was the species that exhibited the shortest reproductive period in the breeding areas near forest remnants, as males called during only two months (Table 1). *Leptodactylus podicipinus* and *Physalaemus cuvieri* called during four and five months, respectively (Table 1). The operational sex ratio in the breeding season was biased towards males in *Eupemphix nattereri*, *Leptodactylus podicipinus* and *Physalaemus cuvieri* with, respectively, 4.7, 3.2 and 1.9 males/female.

Discussion

Our results have demonstrated seasonal variation in anuran body size with larger individuals being registered in the reproductive season than in the dry season. Giaretta and Menin (2004) in a study with *Physalaemus marmoratus* in Minas Gerais state, Brazil, verified similar results for both sexes, the smaller observed sizes were coincident with the end of the reproductive season or the beginning of the dry season. Rossa-Feres, Menin and

Table 1. Temporal distribution of the activity of vocalization of males of three anuran species, in three water bodies near the forest fragments at southeast Brazil.. The abundance was considered as the sum of male in activity of vocalization in the three water bodies.

Species	Nov	Dec	Jan	Feb	Mar
Eupemphix nattereri	17	1			
Leptodactylus podicipinus		5	8	2	2
Physalaemus cuvieri	11	10	3	11	3

Izzo (1999) verified that larger males of *Leptodactylus fuscus* were the first to arrive at reproductive pond at the beginning of rainy season, while small males were found only at the end of rainy season. Diverse studies have shown that recruitment is more important than adult survival in determining population growth (Grafe et al., 2004; Cushman, 2006). In many tropical and sub-tropical anurans, sexual maturity is often reached in the first year especially in males (e.g. Moreira and Lima, 1991; Lampert and Linsenmair, 2002; Richter and Seigel, 2002). Richter and Seigel (2002) verify that adults of *Lithobates sevosus,* in southern Mississippi, are short-lived and that few individuals return to breed in more than one breeding season. Besides, the viability of the population is due to consistent recruitment of juveniles (Richter and Seigel, 2002). Werner et al. (2007) suggest that the majority of turnover events verified to amphibian communities inhabiting 37 water bodies in Michigan, EUA, represent local extinctions. According to authors, the most abundant species are short lived and the majority of individuals in those populations likely only breed once. Therefore, we hypothesized that

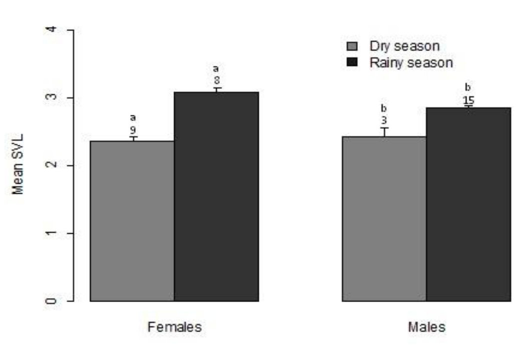

Figure 2. Mean ± SE of snout-vent length (SVL) of males and females of *Physalaemus cuvieri* captured in the rainy season (dark bar) and dry season (light bar). Same letters indicate difference between rainy and dry season (p < 0.05). The numbers above the bars indicate the size of the sample.

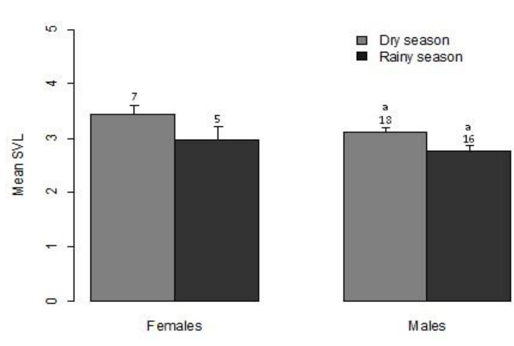

Figure 3. Mean ± SE of snout-vent length (SVL) of males and females of *Leptodactylus podicipinus* captured in the rainy season (dark bar) and dry season (light bar). Same letters indicate difference between rainy and dry season (p < 0.05). The numbers above the bars indicate the size of the sample.

lower individuals captured in the dry season, probably, are juveniles that will be reproductively active adult in the next breeding season, since the sexual maturity is reached in the first year.

As in others studies the operational sex ratio of the three species was skewed toward males (Emlen and Oring, 1977; Pröhl and Hödl, 1999; Pröhl, 2002). According to Emlen and Oring (1977), where OSR is skewed toward males, polygyny is expected, and dominant or larger males are relatively more successful at mating (Höglund, 1989). Prediction that the intensity of male–male competition increases with the operational sex ratio (Emlen and Oring, 1977) probably applies to *E. nattereri*, an explosive breeder with higher OSR in the present study. Rossa-Feres, D. C. (pers. comm.) in a temporary pond, Municipality of Nova Itapirema, São Paulo state, in October 1993, observed three individuals (one female and two males) in amplexus floating in the pond. One of the males (large) was amplexed with the female by the dorsal region and the other male (smaller) was submerged in water and clung to the ventral region of the female body. Both males made movements of kicking with the legs, trying to displace the other. About 3 minutes after starting the observation, the male which was amplexed in the ventral region was displaced by the other that was amplexed on the dorsal region. Similar behavior was registered by Wells (1979) with smallest males of *Rhinella margaritifera* being displaced from amplexus for large ones. The larger body size also influences the spacing between territorial males. The greater distances between neighboring males of *L. fuscus* occurred between large and small males, and small males located more distant from large males than among themselves (Rossa-Feres, Menin and Izzo, 1999).

On the other hand, *Physalaemus cuvieri* e *L. podicipinus* present prolonged breeding period (Martins, 2001; Vasconcelos and Rossa-Feres, 2005; Santos, Rossa-Feres and Casatti, 2007) and males attract females from stationary calling sites (Wells, 1977). Thus, females could potentially use the mating call of a male as an indicator of size (Fairchild, 1981). In anurans, the dominant frequency of the mating call is often negatively correlated with body size (Duellman and Pyles, 1983; Ryan, 1983; Forester and Czarnowsky, 1985). Ryan (1980) has shown that female *Physalaemus pustulosus* preferentially choose mating calls with lower fundamental frequencies and that this is significantly associated with large male size.

Although our data allow only a limited interpretation, we have demonstrated seasonal variation in anuran body size with larger individuals being registered in the reproductive season. Further studies should concentrate on two aspects (a) is larger male and female body size during the breeding season an advantage for all species? (b) Are specimens breeding during a year the same breeding in the next year or are recruited juveniles? The latter aspects can be studied in a mark-recapture program.

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