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ARTICLES

TECHNIQUES

AMPHIBIAN AND REPTILE DISEASES

HERPETOCULTURE

CONSERVATION

HERPETOLOGICAL HISTORY

NOMENCLATURE

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Reproductive Allometry and Sexual Dimorphism in Body Size in the Brazilian Frog *Chiasmocleis leucosticta* (Anura: Microhylidae)

The limited knowledge about the biology and ecology of Neotropical frogs hampers the development of conservation strategies (Brito 2008). Reproductive allometry studies are used to compare biometric parameters of reproductive females and their eggs and/or clutch size (Gayon 2000). Allometric relationships allow estimation of clutch size by assessing female morphology. This information can be useful for a deeper understanding of the evolution of reproductive strategies and population dynamics (Fairbairn 1997). In anurans variation in fecundity is associated with a high diversity of reproductive modes (Duellman and Trueb 1994). Different species sharing the same reproductive mode have similar body sizes and egg diameters (Crump 1982).

The neotropical genus *Chiasmocleis* Méhely, 1904 is the most diverse of the Microhylidae, containing 29 species distributed in Panama and tropical South America, north and east of the Andes (Frost 2015). *Chiasmocleis leucosticta* (Boulenger, 1888) occurs in the Atlantic Forest of southern and southeastern Brazil in the states of Santa Catarina and São Paulo (Frost 2015). This species is an explosive breeder in temporary pools formed in the leaf-litter after heavy rains; eggs are deposited in bubble nests produced by the amplectant pair after egg fertilization (Haddad and Hödl 1997).

Information on the biology of this species is scanty, with few data available on its reproductive biology. In this paper, we investigated the reproductive allometry and tested for sexual dimorphism in body size of *Chiasmocleis leucosticta*.

Fieldwork was carried out each month from September 2008 to January 2010, during a project focused on population dynamics of rodents (Bovendorp 2013), at the Reserva Florestal do Morro Grande, a 9400-ha Atlantic Forest reserve located in the municipality of Cotia, São Paulo state, southeastern Brazil (23°39'–23°48'S, 47°01'–46°55'W). The reserve is covered by a mosaic of secondary forest (ombrophilous and semi-deciduous forests) in different stages of regeneration and areas of mature forest (Metzger et al. 2006). Elevations are between 860 and 1075 m (Veloso 1991) and climate is classified as a Köppen type Cfb, warm temperate and humid (Setzer 1946). Sampling was conducted at three sites along a 6-km linear transect. Each site consisted of a grid composed of 36 pitfall traps (400-ml plastic cups), separated by 20 m and filled with 92% ethanol solution. The traps were originally designed for an arthropod survey. From September 2008 to January 2010, the traps remained open each month for five days, totalling 9180 trap days. A total of 69 individuals of *Chiasmocleis leucosticta* were recovered in the pitfall traps, which were pooled together independently of grid or month of capture.

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On May 2013 all specimens had their snout-to-vent length (SVL) measured with digital callipers (to the nearest 0.5 mm) and were weighed with a digital scale to the nearest 0.002 g. Sex determination was based on the examination of the gonads. Eggs were obtained by dissecting gravid females. The total number of eggs per female was counted, and the diameter of 10 randomly chosen eggs was measured to the nearest 0.001 mm using a Leica stereomicroscope model M205C coupled with a Leica digital camera model DFC295.

We tested the normality of all data through Shapiro-Wilks test, and we log-transformed (base 10) data to assure normality. To test if there was difference in SVL between males and females a two-sample t-Student test was performed. The allometric relationships were tested through simple linear regression (Zar 1999). We tested the relationship between SVL and log number of eggs; SVL and egg diameter; female weight and log number of eggs; female weight and egg diameter; and log number of eggs and egg diameter. All analyses were conducted in the R environment, version 2.12.1 (R Development Core Team 2012).

A total of 69 individuals of *C. leucosticta* were captured in the pitfall traps, including 49 males and 20 females. Male SVL (mean ± SD) was 18.30 ± 2.81 mm (range 12.2–25.3), and female SVL was 23.7 ± 1.58 mm (21.2–27.4); females were significantly larger than males ($t = -10.02$, $df = 59.96$, $P < 0.0001$), as suggested, but not tested, by Cruz et al. (1997). This sexual dimorphism in body size occurs in 90% of anuran species; males usually are larger than females in species that display aggressive behavior (Duellman and Trueb 1994).

The mean female mass was 1.38 ± 0.48 g (0.88–2.97), mean clutch size was 212 ± 7 (101–439), and egg diameter was 1.33 ± 0.13 mm (1.14–1.57) (N = 200). Haddad and Hödl (1997) obtained

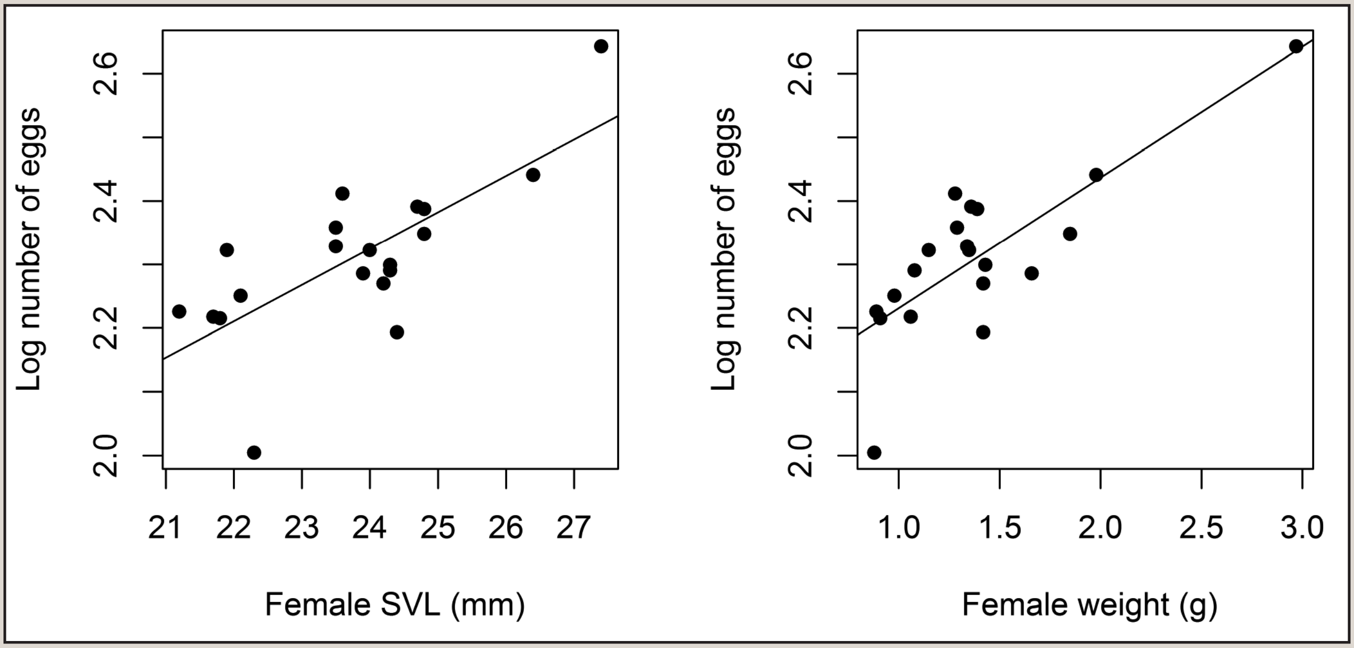

Fig. 1. Relationship between (a) female SVL and number of eggs (Logy = 0.952 + 0.057x; SE: intercept 0.30098, slope 0.01265) and (b) female weight and number of eggs (Logy = 2.025 + 0.205x; SE intercept = 0.05485, SE slope = 0.03755) of *Chiasmocleis leucosticta* (N = 20) from Reserva Florestal do Morro Grande, southeastern Brazil.

similar results for both clutch size (202.6 ± 38.2; 141–233; N = 5) and egg diameter (1.65 ± 0.05 mm; 1.50–1.78; N = 30) in another population of *C. leucosticta* from Ribeirão Branco, in the state of São Paulo, Brazil. Small differences between these two data sets could be attributed to the fact that our measures were taken from ovules rather than from deposited eggs, since after oviposition the capsule swell due to water uptake, increasing egg diameter (Duellman and Trueb 1994). The high similarity between the ovary complement (number of ovules) in the present study and the clutch size obtained by Haddad and Hödl (1997) suggests *C. leucosticta* females deposits their ovarian complement during a single mating, as most anuran species (Duellman 1989). Neotropical microhylids that lay eggs in water (primitive Mode 1 of Duellman and Trueb 1994), like *Chiasmocleis atlantica* (Hartmann et al. 2010) and *Elachistocleis bicolor* (Rodrigues et al. 2003), usually deposit larger clutches composed by smaller eggs. By contrast *Myersiella microps* deposits as few as 25 large (3.25 mm) eggs in terrestrial habitats (Hartmann et al. 2010).

Aquatic oviposition is the primitive condition in anurans, and the evolution of terrestrial reproductive modes involved a decrease in clutch size and an increase in egg diameter (Duellman and Trueb 1994). The increase of egg size was important for the development of terrestrial oviposition, since terrestrial eggs need more yolk in order to support the complete development of the embryo (Pombal and Haddad 2005). The terrestrial reproductive modes in anurans seem to have evolved as a defensive tactic against aquatic predation of eggs (Magnusson and Hero 1991).

The number of eggs was significantly associated with SVL (f = 20.42, $r^2 = 0.53$, $P < 0.001$) and weight (f = 30.01, $r^2 = 0.62$, $P < 0.0001$) (Fig. 1). Egg diameter was not significantly related to SVL (f = 3.11, $r^2 = 0.15$, $P = 0.09$), weight (f = 0.71, $r^2 = 0.04$, $P = 0.40$) or number of eggs (f = 3.93, $r^2 = 0.17$, $P = 0.06$). The positive relationships between number of eggs and female SVL and female weight have already been described for other anuran species (Duellman and Trueb 1994; Hartmann et al. 2010). The number of eggs produced by a female increases with body volume (Jorgensen 1992). However, environmental factors such as temperature and food availability might reduce or stimulate egg production (Kaplan 1989).

Accurate fecundity estimates are important for demographic models of amphibian conservation used in population viability analyses (e.g., Di Minin and Griffiths 2011). Although our results have differed only slightly from data obtained by Haddad and Hödl (1997) for another population of *C. leucosticta*, many anuran species are known to exhibit differences in fecundity between populations from different altitudes (Morrison and Hero 2003).

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