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Reproductive biology of *Philodryas olfersii* (Serpentes, Dipsadidae) in a subtropical region of Brazil

Paulo Cesar Mattos Dourado de Mesquita[1], George Lucas Sá-Polidoro[2] & Sonia Zanini Cechin[1]

[1]*Programa de Pós-Graduação em Biodiversidade Animal, Universidade Federal de Santa Maria, Avenida Roraima, 1000. CEP 97105-900, Santa Maria, Rio Grande do Sul, Brazil,*

[2]*Laboratório de Herpetologia, Departamento de Biologia, Universidade Federal de Santa Maria. Avenida Roraima, 1000. CEP 97105-900, Santa Maria, Rio Grande do Sul, Brazil*

We studied the reproductive biology of a subtropical population of *Philodryas olfersii* through the analysis of 263 museum specimens. Females were found to have larger bodies with males having longer tails. Testicular volume has a tendency to decrease in colder months but histological analyses are still needed to validate the macroscopic evidence. In females, secondary follicles were found throughout the year but ovulation was restricted to three months during the warmer period. Clutch size was correlated with female size and varied between 4 and 11 eggs, with a mean relative clutch mass of 0.187. Ovigerous females frequently also presented secondary follicles, and potential fecundity was significantly higher than real fecundity. We suggest that estimates of clutch size based on the number of vitellogenic follicles may be inappropriate. In addition, although some patterns of the reproductive biology of *Philodryas olfersii* were similar to other members of the tribe Philodryadini, we found important differences such as shorter reproductive season of females, indicating that phylogenetical traits and environmental conditions influence aspects of the life history of the species.

Key words: Life history, Philodryadini, reproduction, snakes

INTRODUCTION

Reproductive biology is a central theme for understanding the evolution of strategies in the natural history of snakes, because, ultimately, the most important method for the perpetuation of a species is reproductive success (Seigel & Ford, 1987; Holycross & Goldberg, 2001).

It is generally considered that all snakes in temperate regions reproduce seasonally and tropical snakes present a greater diversity of reproductive patterns (Greene, 1997; Pizzatto et al., 2007a; Pinto et al., 2010). Classically, the reproductive cycles of snakes were classified as continuous or discontinuous (see Saint-Girons, 1982). However, recent advances demonstrated that a truly continuous cycle is largely absent in snakes, and the reproductive cycles of almost all species can be considered "seasonal" to some extent (Almeida-Santos et al., 2006; Mathies, 2011). Subtropical environments in South America may resemble temperate regions with regards to seasonal changes in temperature, or tropical environments in terms of environmental heterogeneity and species composition. That makes these environments particularly interesting for the investigation of patterns of reproductive cycles and the effects of climate and environment on the reproductive biology of snakes.

Despite increased efforts to study the reproductive biology of snakes, subtropical populations of most species have still received little attention (see Pontes & Di-Bernardo, 1988; Pizzatto et al., 2008; Leite et al., 2009a). The green snake, *Philodryas olfersii*, is a medium-sized Dipsadidae widely distributed in South America (Peters & Orejas-Miranda, 1973). Although there is a relatively large amount of information available on for example diet and habitat use (e.g., Vitt, 1980; Cechin & Hartmann, 2001; Hartmann & Marques, 2005; Leite et al., 2009b), information on its reproductive biology is restricted to tropical areas and only anecdotal reports are available from subtropical populations (Pontes & Di-Bernardo, 1988; Fowler & Salomão, 1995; Molina & Rocha, 1996; Fowler et al., 1998; Sawaya et al., 2008; Mesquita et al., 2012). Herein, we describe some aspects of the reproductive biology of *Philodryas olfersii* in a subtropical environment in Brazil.

MATERIALS AND METHODS

All specimens were collected in the central region of the State of Rio Grande do Sul, Brazil. According to the classification proposed by Rossato (2011), the climate type is Subtropical II, with influence of polar systems and continentality. The mean temperature of the coldest month is 14.1°C (July) and the mean temperature of the warmest month is 24.8°C (January). Absolute minimum temperatures are lower than -2°C and absolute maximum temperatures are over 40°C. Rainfall is evenly distributed during the year and ranges between 1500–1750 mm.
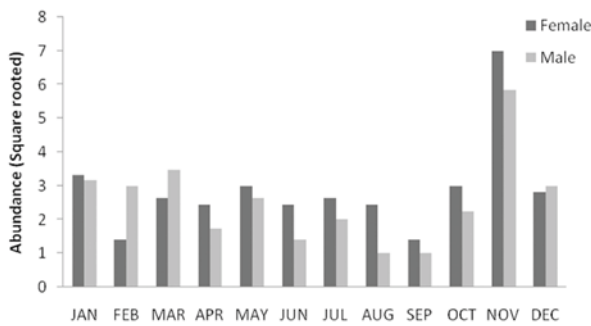
Correspondence: P.C.M.D. Mesquita (paulocmdm@gmail.com)

Fig. 1. Monthly abundance (square root transformed) of male and female *P. olfersii* in a subtropical environment in Brazil.

We analyzed 263 specimens from subtropical populations deposited on the Collection of Herpetology of the Federal University of Santa Maria, state of Rio Grande do Sul, Brazil. For each individual, we obtained information on sex, month of capture, snout-vent length (SVL; mm), tail length (TL; mm), head length (HL; mm), mass after the drainage of preservative liquids (g) and the condition, mass, number and size of the vitellogenic follicles or eggs (females), or the condition and volume of the testes and diameter of the distal portion of the deferent ducts (males).

Analysis
Seasonal activity was inferred through the monthly abundance of males and females. To evaluate whether there was activity synchrony among males and females we performed a correlation test with the values of number of individuals transformed to Ln(n)+1, where n is the number of individuals. Unsexed individuals or those with no information on the date of capture were excluded from this analysis.

Sexual dimorphism was evaluated based on SVL, TL, HL and mass. We compared SVL between males and females using a *t*-test and a measure of sexual dimorphism (SSD, Shine, 1994). SSD was calculated as SSD=(mean SVL of the larger sex/mean SVL of the smaller sex)-1. SSD is positive if females are larger, and negative if males are larger.

We compared TL and HL through analysis of covariance (ANCOVA) using SVL as a covariate. Similarly, to assess sexual differences in mass we used ANCOVA with total length (SVL+TL) as a covariate of the cubic root of the mass. Whenever necessary the measurements were transformed to their natural logarithms to fill the assumptions of the ANCOVA.

Specimens with a total or partial amputation of the tail were excluded from the analyses that used measures of TL and total length. Individuals whose sex was not determined, because of museum restrictions or poor conservation of internal organs, were excluded from all analyses of sexual dimorphism. We included immature individuals in the sexual dimorphism analyses because the number of specimens evaluated was large enough so that the graphical representation could indicate whether the dimorphism was related to sexual maturity (Mesquita et al., 2011).

Males were considered mature when entangled and opaque deferent ducts were presented. Testicular volume was estimated as V=(3/4)πabc, where a
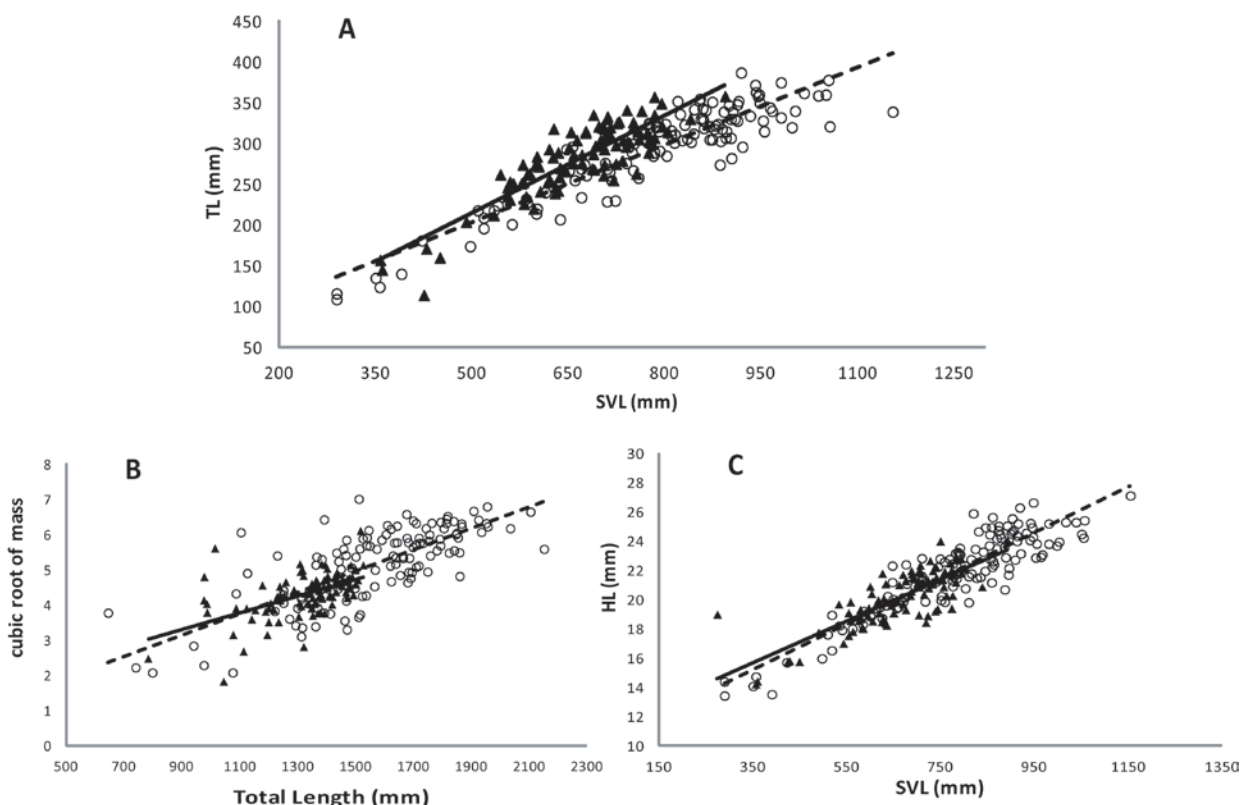

Fig. 2. Sexual dimorphism in (A) tail length, (B) cubic root of mass and (C) head length *Philodryas olfersii*. Triangles and full lines (males); circles and dotted lines (females).

represents length, b represents the larger diameter and c represents the smaller diameter of the testes. We assessed monthly variation on testicular volume and deferent ducts diameter through analysis of variance (ANOVA). Because testicular volume can be affected by SVL, we used the residuals of the regression as the measure of volume because it can be related to patterns of sperm production in males (Shine, 1977).

Females were considered mature when enlarged and yellowish ovarian follicles were present or when there were oviductal eggs (Shine, 1977). The reproductive cycle was determined based on the monthly variation of the size of vitellogenic follicles and the presence of eggs in the oviducts. We evaluated variation in follicle length through ANOVA and Tukey's pairwise test.

We analyzed fecundity based on the number of follicles and the number of eggs found in the oviducts. Potential fecundity was determined by the number of secondary follicles, and real fecundity was determined by the actual number of eggs. Potential versus observed fecundity was compared through a t-test. The association between female SVL and number of eggs, as well as the association of female mass with egg mass were analyzed through linear correlation. Reproductive investment was defined as the ratio between female mass and egg mass (Seigel & Ford, 1987; Shine, 1992).

RESULTS

We obtained information on the month of capture for 97 males and 123 females. Individuals were found during every month of the year, with a strong peak in November for both sexes (Fig. 1). Males and females presented synchrony in activity during the year (r^2=0.65; p=0.02). Female SVL varied between 289 mm and 1155 mm (mean=783.35±164.46; n=126) and male SVL varied between 357 mm and 894 mm (mean=668.11±98.25; n=97). The smallest reproductive female presented an SVL of 637 mm and the smallest reproductive male had an SVL of 491 mm. SSD was 0.17, and females were significantly larger than males (ANOVA, $F_{1, 223}$=38.69; p<0.001).

Males had longer relative tail lengths than females (ANCOVA, $F_{1, 216}$=60.27; p<0.001) (Fig. 2A) without significant sexual dimorphism in mass (ANCOVA, $F_{1,223}$=2.81; p=0.095; Fig. 2B) and head length (ANCOVA, $F_{1,222}$=0.001; p=0.978; Fig. 2C).
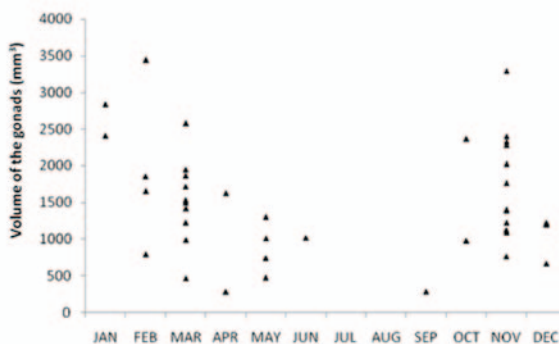
Reproductive females measured between 637 and 1155 mm SVL (mean=868.06±99.70). Clutch size (number of oviductal eggs) varied between 4 and 11 (mean=7.17±1.51; n=59). Many ovigerous females presented eggs and enlarged follicles simultaneously, and the number of vitellogenic follicles varied between 3 and 22 (mean=8.51±3.56; n=58). The potential fecundity was significantly higher than the observed fecundity (t=-2.41; p=0.017). There was a positive correlation between female SVL and clutch size (R^2=0.271; p=0.033). Reproductive investment (or relative clutch mass) varied between 0.046 and 0.456 (mean=0.187±0.077).

We found no significant monthly variation on testicle volume (ANOVA F=1.63, p=0.12) and deferent duct diameter (ANOVA F=1.38, p=0.20), despite a tendency for reduction during the winter months (Fig. 3).

Follicles in secondary vitellogenesis were registered throughout the year, but ovulation and oviposition appeared to be restricted to November–January (Fig. 4). Follicle size differed between months, particularly between November and December ($F_{106, 11}$=5.769; p<0.0001).

DISCUSSION

We observed a uni-modal pattern of activity with a peak during the warmer months. The peak of activity coincides with the period when most reproductive individuals were found, suggesting that the breeding season is of major importance for the seasonal activity of *P. olfersii*. Similar patterns were observed for a subtropical population of *P. patagoniensis* and also a tropical population of *P. olfersii*, while other tropical congeners seems to attain longer peaks of activity, indicating that not only reproduction, but also climate, food availability and possibly some degree of phylogenetic conservatism may influence their seasonal activity pattern (Fowler & Salomão, 1994a; Marques et al., 2001; Lopez & Giraudo, 2008; Marques et al., 2006; Mesquita et al., 2011).

The patterns of sexual dimorphism in subtropical *P. olfersii* are common for most Xenodontinae, although the dimorphism in tail length is less frequent in arboreal and semi-arboreal species (Pizzatto et al., 2007b). Here, we provide evidence for sexual dimorphism in TL, which is different to the findings of Fowler & Salomão (1994b). Reproductive and evolutionary constraints as well as the
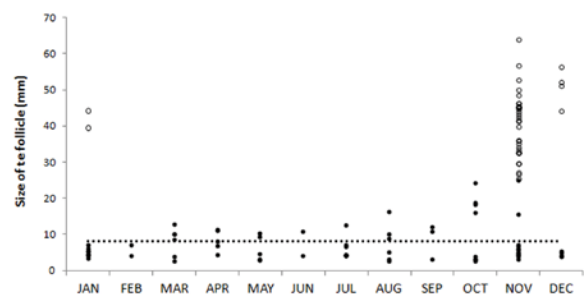

Fig. 3. Monthly variation on the volume of the largest testicle of *Philodryas olfersii*.

Fig. 4. Monthly variation of the size of the largest follicle (black circles) or eggs (white circles) of *Philodryas olfersii*. Dotted line indicates the size of the smallest secondary follicle (8.06 mm).

degree of phylogenetic conservatism may be higher in subtropical conditions than in tropical environments (Webb et al., 2003). It is furthermore unclear whether the effect of body size on TL was controlled for the analysis used by Fowler & Salomão (1994b).

Our results indicate that sexual dimorphism in TL is related to sexual maturity, because such a difference is not evident in juveniles (see also Mesquita et al., 2011, with *P. nattereri*). As expected, males mature at a smaller size than females, which may be related to the smaller range of sizes in males (Shine, 1978; Parker & Plummer, 1987). The difference between potential fecundity and real fecundity indicate that reproduction in *Philodryas olfersii* is constrained rather than maximized (Shine, 1992). Thus, the usual method to estimate clutch sizes based on the number of follicles during secondary vitellogenesis may be inappropriate for some species (Shine, 1994; Cappellari et al., 2011).

Although individuals of *P. olfersii* can be found active throughout the year, the reproductive cycle is not continuous. Males seem to present a period of reproductive dormancy during the coldest months (June–September) and although females presented vitellogenic follicles throughout the year, ovulation is clearly seasonal and restricted to November–January. The observation of a period of apparent reproductive latency in males during winter is a common pattern for most reptiles of temperate regions, and also for some from subtropical areas (Pizzatto et al., 2007a; Capellari et al., 2011). However, it contrasts with the observed variation in testes volume and deferent duct diameter, consistent with the ideas of Mathies (2011) that variation of macroscopic structures is not always a good indicator of spermatogenesis, and that histological analysis is the only reliable method to describe the reproductive cycle of male snakes (see Almeida-Santos et al., 2006). Our results are also consistent with Mathies (2011) who states that the mere observation of vitellogenic follicles cannot be considered as indicator of imminent ovulation in snakes, because the period of vitellogenesis is highly variable among species, even when seasonal patterns of egg production are evident (see Hartmann et al., 2004; Lopez & Giraudo, 2008; Marques et al., 2006).

Despite evidence that reproductive traits are relatively conservative in tropical snakes (Saint-Girons, 1982; Pizzatto et al., 2008), the period of egg production of only three months is the shortest reproductive period registered for the tribe Philodryadini (Vitt, 1980; Fowler et al., 1998; Marques et al., 2006; Lopez & Giraudo, 2008; Oliveira, 2008; Mesquita et al., 2011), and may therefore be a result of environmental pressures. The only Philodryadini with a similarly short reproductive period is *Tropidodryas serra*, for which the evidence is based on only three clutches (Oliveira, 2008). Many other Squamata from subtropical areas also present seasonal cycles with short periods of ovulation that may also be related to environmental constraint (Pontes & Di-Bernardo, 1988; Hartmann et al., 2004; Balestrin & Di-Bernardo, 2005; Leite et al., 2009a; Zanella & Cechin, 2010; Cappellari et al., 2011).

The activity of snakes is greatly affected by low temperatures (Lillywhite, 1987), and the amount of energy allocated for reproduction in areas with cold winters is constrained by physiological limitations inherent to ectothermy (Zanella & Cechin, 2010). For example, Pinto & Fernandes (2004) found important differences in the reproductive biology of populations of *Liophis poecilogyrus* from different latitudes, and the results of our study are consistent with the idea that different climatic conditions influence reproductive patterns.

The simultaneous presence of eggs and follicles in secondary vitellogenesis suggest the possibility of multiple clutches during one reproductive season, which has been recorded for the congener *P. nattereri* (Mesquita et al., 2011). Differently from what was suggested by Molina & Rocha (1996), *P. olfersii* females have the potential to have multiple clutches. In fact, Cechin & Hartmann (2001) collected a female in courtship behaviour in March. We reanalyzed this specimen and found a loose oviduct, providing evidence for recent oviposition and suggesting that *P. olfersii* is capable of multiple matings and multiple clutches during a single reproductive period.

We found a wide range of relative clutch mass, which was however largely due to extreme values. We suggest that low RCM occurs in few females of high fitness, and may represent a second event of ovulation within one reproductive season. Based on three clutches of *Philodryas olfersii*, Molina & Rocha (1996) presented RCM within our range.

Our results show that some traits of the reproductive biology of *Philodryas olfersii* are phylogenetically conservative, such as the relatively high number of vitellogenic follicles in the tribe Philodryadini (Vitt, 1980; Fowler et al., 1998; Marques et al., 2006; Lopez & Giraudo, 2008; Oliveira, 2008; Mesquita et al., 2011). The ability to simultaneously produce eggs and vitellogenic follicles was also observed for the congener *P. nattereri* (Mesquita et al., 2011), and the size of sexual maturation almost matches tropical populations of *P. olfersii* (Fowler & Salomão, 1995). It is therefore plausible that the reproductive potential that is not fully converted in clutches arises from phylogenetic conservatism, but the duration of the reproductive period may be constrained by environmental factors.

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