

Reproductive biology of *Philodryas patagoniensis* (Snakes: Dipsadidae) in south Brazil: Female reproductive cycle

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Abstract

In this study, we describe the female reproductive cycle of *Philodryas patagoniensis* in south Brazil, which was described through morpho-anatomical and histological analyses. The peak of secondary vitellogenesis occurred during winter–spring (July–December), ovulation in spring (October–December), mating and fertilization in spring–summer (October–February), oviposition in spring–autumn (October–May) and births from late spring to autumn (December–July). The diameter of vitellogenic follicles/eggs was larger in winter–spring than in other seasons. The diameter of the shell glands was also larger in winter–spring. In spite of the clear reproductive peak, gonads only showed reduced activity in the autumn. Therefore, at the individual level, females have a discontinuous cyclical reproduction; in the populational level, the reproductive cycle is seasonal semisynchronous. We support the hypothesis that *P. patagoniensis* have the ability to produce multiple clutches with long-term stored sperm. Sexual dimorphism in body size was evident, and females are significantly larger and heavier than males. Larger females were able to produce follicles and eggs in larger amount and size. The maternal body size was positively related to the reproductive effort and fecundity. To conclude, we deliberated about the proximal and distal causes that influence the reproductive traits and patterns of *P. patagoniensis*.

KEYWORDS

reproduction, sexual dimorphism, sperm storage, vitellogenesis

1 | INTRODUCTION

The life-history theory attempts to describe how the natural selection and evolution compel organisms to improve their “Darwinian fitness” (Stearns, Ackermann, Doebeli, & Kaiser, 2000). The fitness is related to the reproductive success; however, it is not a cheap endeavour. Physiological costs of fitness may be translated into negative relationships between two life-history traits, which are known as “trade-offs” (Ford & Seigel, 1989). Therefore, to understand why and how snakes use the limited energy and available resources, we need to analyse many “trade-offs” involved in maximizing

the survival and reproduction and, consequently, optimize the fitness (Fabian & Flatt, 2012).

The life-history strategies of an organism are directly related to the adaptation to local conditions (Stearns et al., 2000). However, studies on snakes’ reproduction have extensively examined a small number of temperate species, while the neotropical species have received more attention in the past two decades (Almeida-Santos et al., 2014). Therefore, the reproductive information about a large number of species is required to understand the evolution of reproductive traits and patterns (Barros, Sueiro, & Almeida-Santos, 2012).

In snakes, many aspects of the female reproductive cycle occur independently of the male cycle (Taylor & DeNardo, 2011). In general, females' cycle tends to be less variable than males, and the vitellogenesis, ovulation, and oviposition are the most important events used to classify the reproductive cycle (Aldridge, Goldberg, Wisniewski, Bufalino, & Dillman, 2009). Furthermore, these events may drastically diverge depending on evolutionary history, temperature, photoperiod, duration of reproductive season, food availability, body condition and quality of fat reserves (Bellini, Arzamendia, & Giraudo, 2016; Naulleau & Bonnet, 1996). Summarizing the plasticity in life-history traits is influenced by environmental factors as well as by genetic characteristics (Seigel & Ford, 2001). Squamates can exhibit variation in reproductive tactics even in the intraspecific level (Barros et al., 2012).

An important event used to classify reproductive cycles is the seasonal association of mating and gonadal activity (Crews, 1984). Briefly, species with associated reproductive pattern mate when the gonadal activity is elevated, while species with dissociated reproduction mate when gonads are quiescent. In snakes with dissociated cycle, the timing of mating and fertilization are asynchronous and a sperm-storage mechanism is required so fertilization can be delayed (Schuett, 1992).

Female sperm-storage structures evolved independently for different times in Squamate (Sever & Hamlett, 2002). Many explanations are proposed for this feature as follows: dissociated reproductive events, scarcity of sexual partners, female ability of laying more than one egg clutch in the same reproductive season, less predation risk linked to the reproductive endeavours and sexual selection through sperm competition (Almeida-Santos & Salomão, 1997; Birkhead & Moller, 1993; Schuett, 1992; Tryon, 1984).

Philodryas patagoniensis (Girard, 1858) is a diurnal terrestrial species (Hartmann & Marques, 2005) widely distributed in savannahs and grasslands of Brazil, Bolivia, Paraguay, Argentina and Uruguay (Peters & Orejas-Miranda, 1970). They are oviparous snakes that reproduce seasonally. Studies on female reproductive cycle have been performed on Brazilian (Fowler, Salomão, & Jordão, 1998; Pontes, 2007; Rojas, Barros, & Almeida-Santos, 2015) and Argentinean (López & Giraudo, 2008) populations, although just Rojas et al. (2015) explored the histology of the female oviduct.

Therefore, the present study describes the reproductive biology of *P. patagoniensis* females in south Brazil through morpho-anatomical and histological analyses, testing the following hypothesis: (i) the species should have a seasonal pattern of reproduction in the subtropical region; (ii) *P. patagoniensis* females should be able to produce multiple clutches in the same reproductive season; (iii) the oviduct of *Philodryas patagoniensis* must have sperm-storage

adaptations; and (iv) the female-biased sexual dimorphism in body size may be related to fecundity selection in this species.

2 | METHOD

2.1 | Data collection

We analysed individuals of *P. patagoniensis* from the south region of Brazil (Rio Grande do Sul, Paraná and Santa Catarina States) available in the herpetological collections of the of the Santa Maria Federal University (ZUFMS) and Pontifical Catholic University of Rio Grande do Sul (MCP-PUCRS) (Appendix).

The climate in south Brazil is classified as humid subtropical (Cfa and Cfb) according to Köppen's climate classification. Rainfall is well distributed throughout the seasons (mean annual precipitation ranging from 1,000 to 2,000 mm). Therefore, the region shows a well-defined temperature seasonality (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013).

Reproductive events were described according to austral seasons: summer (late December–late March), autumn (late March–late June), winter (late June–late September) and spring (late September–late December). The reproductive cycle of mature females was analysed considering morpho-anatomical and histological changes in ovary and oviduct (Rojas et al., 2015).

We analysed 112 *P. patagoniensis* females. For each individual, we obtained information concerning month of death, snout–vent length (SVL) and body mass (BM). We evaluated the number of vitellogenic follicles and/or eggs and the diameter of the largest follicle and/or oviductal eggs as the morpho-anatomical variables. To investigate size–fecundity relationships and reproductive investment, we measured the ovary mass (follicles) and calculated the Gonadosomatic Index (GSI; Clesson, Bautista, Baleckaitis, & Krohmer, 2002).

To investigate sexual dimorphism, we measured 57 males and 46 females (mature individuals) according to the following external morphological traits: snout–vent length (SVL), tail length (TL) and body mass (BM). We calculated the degree of sexual dimorphism (SSD) in SVL using the formula proposed by Shine (1994). We considered mature males larger than 440 mm of snout–vent length and 690 mm of total length (Loebens, Cechin, Theis, Mouras, & Almeida-Santos, 2016). Females' sexual maturity was determined using the presence of spermatozoa in the vagina or oviduct (post-copulating) and/or the presence of mature follicles in ovary or eggs in oviduct as the main criterions. The aspect of the oviduct, either with folds or loose (post-spawning period), was used as a secondary criterion for stablishing females sexual maturity (Shine, 1977).

2.2 | Histology

We dissected 55 females to obtain histological samples from the following structures: infundibulum (anterior and posterior), uterus, utero-vaginal junction (UVJ) and vagina (Rojas et al., 2015). As a standard procedure, only right-side organs were used in this study. The tissue samples were processed for light microscopy using historesin method. Sections were cut at 2 μm thick in a Leica RM2245 microtome. Slides were analysed using a ZEISS Axio Scope.A1 microscope with an Axiocam MRc5 camera.

The presence of spermatozoa and sperm-storage glands/tubules was verified through different regions of the oviduct. Females with sperm in the vagina were considered as recent-mating data (Sever, Ryan, Morris, Patton, & Swafford, 2000) because direct evidence of mating events (e.g. observations in the wild) is not available. We also evaluated the seasonal variation in the shell glands (uterus) diameter (Siegel & Sever, 2008). We obtained 10 measures of this microscopic variable from each individual.

2.3 | Statistical analysis

The reproductive cycle was determined based on the seasonal variation in the diameter of vitellogenic follicles/eggs and shell and GSI values. These variables were investigated by analysis of variance (ANOVA) and significant results examined by post hoc tests (Tukey).

To investigate size-fecundity relationships, we used linear regressions between the following variables: BM vs. ovary mass, BM vs. number of follicles/eggs and BM vs. follicle/egg diameter. Correlations between body size (SVL) and values of GSI were tested through Pearson correlation coefficient. The association between female SVL and clutch size (number of oviductal eggs) was analysed through linear regression.

The fecundity was analysed comparing the potential fecundity (number of secondary follicles) and the observed fecundity (number of eggs in the oviducts) through a *t* test. We investigated the relationship between follicles/eggs number vs. follicles/eggs size using Pearson correlation analysis.

To analyse sexual dimorphism, we compared SVL of both sexes using analysis of variance (ANOVA). Because TL and BM vary with body length, we used SVL as a covariate in the analysis of covariance (ANCOVA) to compare these variables between the sexes (Shine, 1994).

3 | RESULTS

3.1 | Vitellogenic cycle

The diameter of vitellogenic follicles/eggs exhibited an increase during winter and spring ($F = 11.47$; $p < .00001$; Table 1; Figure 1a). Follicles/eggs were larger during winter and spring when compared with autumn ($p < .005$ in both) and summer ($p < .05$ in both). The GSI differed among the seasons ($F = 6.26$; $p < .005$; Table 1; Figure 1b) and ovaries were heavier in winter and spring when compared with autumn ($p < .05$ in both) and summer ($p < .05$ in both). Considering the microscopic data, variation in the diameter of the shell glands was seasonal ($F = 7.80$; $p < .005$; Table 1; Figure 1c) and it was larger in winter and spring when compared with summer ($p < .01$ and $p < .05$; Table 1). Therefore, the shell glands in the uterus of *P. patagoniensis* should be atrophied during summer–autumn (Figure 2a,b) and hypertrophied during winter–spring (Figure 2c,d).

Follicles in reproductively active females were larger than 11.71 mm. Females with vitellogenic follicles were found in all the seasons; despite this, vitellogenesis was more evident in the period between late winter and spring (July–December), and ovulation in spring (October–December). Females with sperm in the vagina (Figure 3a) were recorded in spring and early summer (October, $n = 2$; November, $n = 1$; December, $n = 2$; January, $n = 3$; and February, $n = 2$). Ovigerous females ($n = 26$) were recorded from spring to early winter (October, $n = 9$; November, $n = 8$; December, $n = 3$; January, $n = 1$; February, $n = 2$; March, $n = 1$; April, $n = 1$; and July, $n = 1$). Females simultaneously presenting mature follicles and eggs were recorded from spring to autumn (October, $n = 2$; November, $n = 5$; December, $n = 1$; January, $n = 1$; February, $n = 2$; March, $n = 1$; and April, $n = 2$). Egg clutches ($n = 13$)

TABLE 1 Macroscopic and microscopic measurements of *Philodryas patagoniensis* females in south Brazil

Season	SVL (mm)	GSI	Follicles diameter (mm)	Shell glands (μm)
Summer (A)	844.93 \pm 39.72	0.57 \pm 0.09 ^{C,D}	8.44 \pm 1.73 ^{C,D}	24.51 \pm 2.08 ^{C,D}
Autumn (B)	859.50 \pm 29.83	0.73 \pm 0.12 ^{C,D}	5.95 \pm 0.37 ^{C,D}	31.96 \pm 4.89
Winter (C)	919.86 \pm 53.37	3.54 \pm 1.05 ^{A,B}	12.27 \pm 2.09 ^{A,B}	42.79 \pm 4.50 ^A
Spring (D)	851.18 \pm 18.25	3.72 \pm 0.94 ^{A,B}	11.73 \pm 0.99 ^{A,B}	43.89 \pm 3.25 ^A

SVL, snout–vent length; GSI, gonadosomatic index.

Post hoc analysis: significant differences ($p < .05$) between seasons are indicated by letters. Each letter represents a season. Data are expressed as mean \pm standard errors.

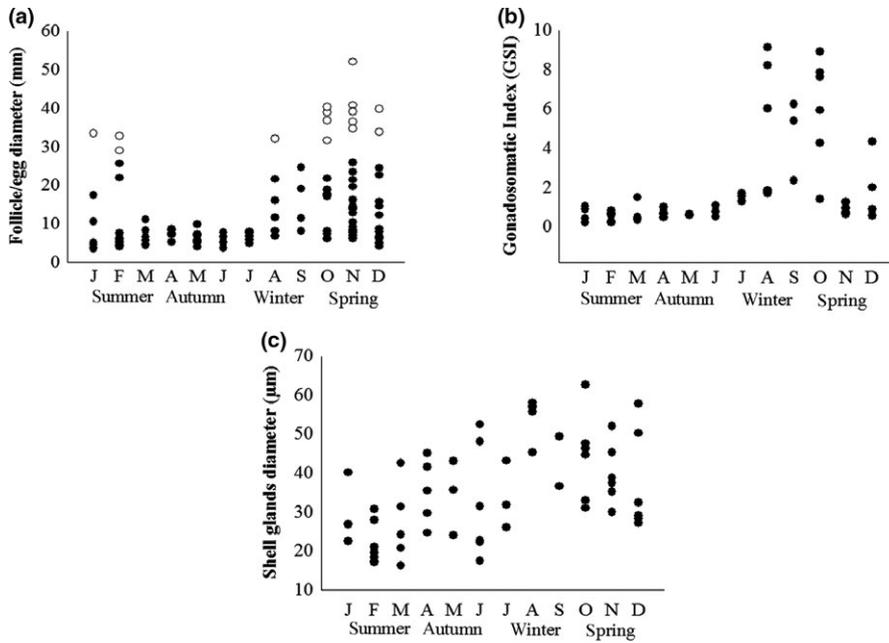


FIGURE 1 Seasonal variation in (a) follicles/eggs diameter, (b) gonadosomatic index and (c) shell glands diameter of *Philodryas patagoniensis* in south Brazil. (●): vitellogenic follicles. (○): eggs

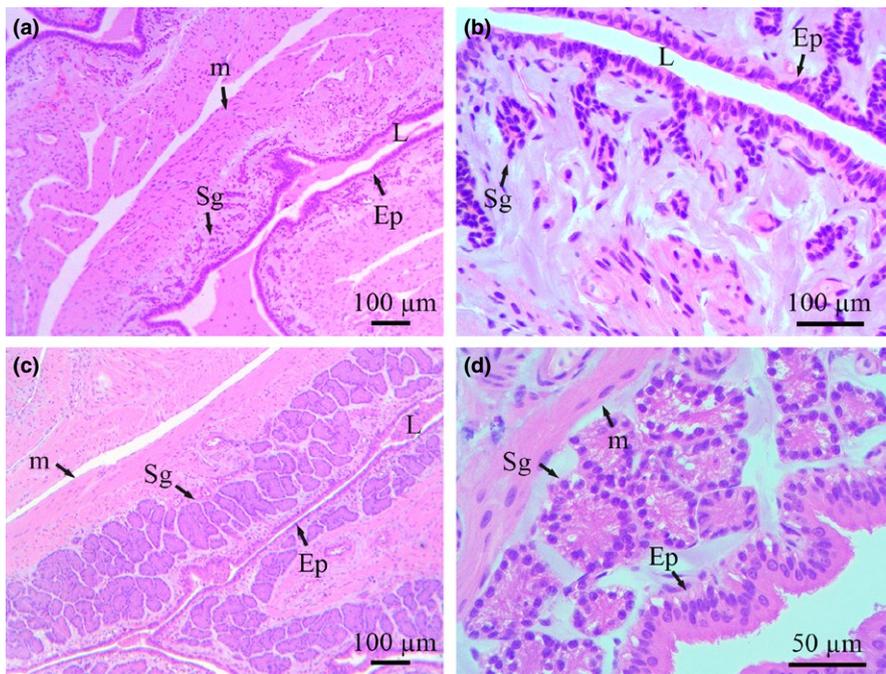


FIGURE 2 Transverse sections of *Philodryas patagoniensis* uterus showing shell glands in atrophy during summer–autumn (a, b), and hypertrophy during winter–spring (c, d). Ep, epithelium; Sg, shell gland; M, muscularis; L, lumen. [Colour figure can be viewed at wileyonlinelibrary.com]

were recorded from spring to early autumn (October, $n = 1$; November, $n = 5$; December, $n = 2$; January, $n = 2$; February, $n = 1$; March, $n = 1$; and May, $n = 1$). Neonates were recorded from late spring (December) to early winter (July).

3.2 | Sperm storage

In *P. patagoniensis*, we could identify sperm storage in two different locations of the oviduct: UVJ and posterior infundibulum. Sperm storage occurred in pregnant

females and in those with primary and secondary vitellogenic. Females with sperm in deep furrows (crypts) of the UVJ (Figure 3b) were recorded throughout the year (January, $n = 2$; April, $n = 1$; June, $n = 2$; July, $n = 2$; August, $n = 4$; October, $n = 3$; November, $n = 3$; and December, $n = 1$). The furrows in UVJ were filled with undulating parallel arrays of sperm tails. We could also identify sperm-storage receptacles with sperm debris in the posterior infundibulum (Figure 3c,d) mainly in spring and summer (November, $n = 3$; January, $n = 1$; February, $n = 2$; and June, $n = 1$).

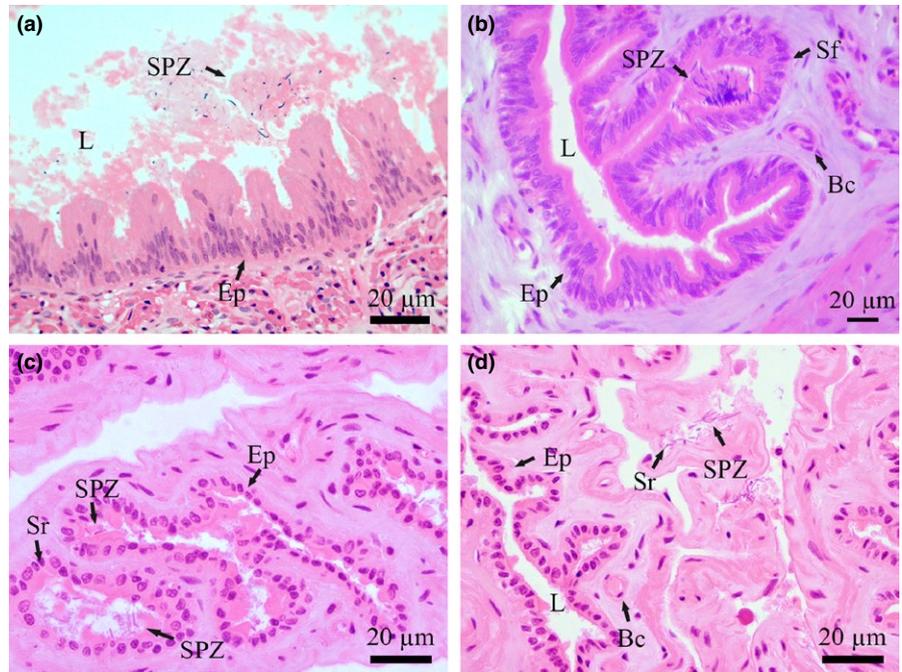


FIGURE 3 Transverse sections of the UVJ of *Philodryas patagoniensis* exhibiting sperm storage in furrows/glands (a), vagina with post-copulating sperm (b) and the posterior infundibulum exhibiting sperm-storage receptacles (c, d). Ep, epithelium; bc, blood capillaries; L, lumen; Spz, spermatozoa; Sf, sperm-storage furrows; Sr, sperm-storage receptacles. [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Size-fecundity relationships

BM had a significant effect ($r = .48$; $p < .005$) and a positive relationship ($R^2 = .21$; $F = 15.12$; $p < .0005$) with the ovary mass (Figure 4a). The mean ovary mass was 6.07 ± 9.84 g (range of 0.12–44.49 g). The BM had a weak but significant relationship with the number of follicles/eggs ($R^2 = .07$; $F = 5.13$; $p < .05$; Figure 4b). The mean clutch size (number of eggs in the oviducts) was 12.81 ± 0.90 (range of 5–22), while the average number of secondary follicles in ovary was 18.40 ± 1.18 (range of 6–40). There was a positive correlation between female SVL and clutch size ($R^2 = .43$; $F = 18.45$; $p < .0005$; Figure 4c). The diameter of follicles/eggs showed a positive relation with BM ($R^2 = .16$; $F = 10.76$; $p < .005$; Figure 4d). The average length of secondary follicles was 16.61 ± 8.55 mm (range of 11.71–25.71) and oviductal eggs measured in average 36.02 ± 6.51 mm (range of 23.60–51.94). The overall mean of the GSI of females was 2.12 ± 0.21 (range of 0.19–9.10). There was a significant positive correlation between female's SVL and reproductive effort (GSI; $r = .36$; $p < .01$; Figure 4e).

The potential fecundity (secondary follicles) was significantly higher than the observed fecundity (eggs; $t = 3.54$; $p < .005$; Figure 5a). The follicle/egg number was negatively related to the follicle/egg size ($r = -.44$; $p < .0001$; Figure 5b).

The degree of SSD was 0.37, indicating that the mean SVL of mature females was 36.86% larger than that of mature males. The SVL of mature females ranged from 500 to 1,480 mm ($\bar{x} = 915.02$), TL from 147 to 410 mm ($\bar{x} = 267.87$), total length from 810 to 1,447 mm ($\bar{x} = 1,156.49$) and BM from 117.9 to 604 ($\bar{x} = 262.17$).

The SVL of the mature males ranged from 440 to 840 mm ($\bar{x} = 668.57$), TL from 150 to 370 mm ($\bar{x} = 279.55$), total length from 690 to 1,180 ($\bar{x} = 948.15$) and BM from 61.1 to 354 ($\bar{x} = 117.53$). Sexual body size dimorphism was evident in mature individuals, being females significantly larger ($F = 97.72$; $p < .0001$) and heavier ($F = 8.35$; $p < .005$) than males (Figure 6a). However, males presented longer TL than females ($F = 10.23$; $p < .005$ Figure 6b).

4 | DISCUSSION

The reproductive cycle of *P. patagoniensis* females in south Brazil is characterized by the following characteristics. The peak of secondary vitellogenesis occurs from winter to spring (July–December), ovulation in spring (October–December), mating and fertilization in spring and early summer (October–February), oviposition from spring to autumn (October–May), and births from late spring to early winter (December–July). The extended period of recruitment was probably recorded due to the occurrence of two birth seasons that may have happened successively. The first birth season occurs from late spring to summer (December–March), while the second season may occur from autumn to early winter (April–July). This estimate was made considering the ability of females to produce multiple clutches with long-term stored sperm.

Philodryas patagoniensis exhibited a prolonged period in which females were ovigerous and had similar size distributions of vitellogenic follicles; therefore, gonads only revealed reduced activity in autumn. Therefore, we can summarize the reproductive cycle of the species according to Mathies

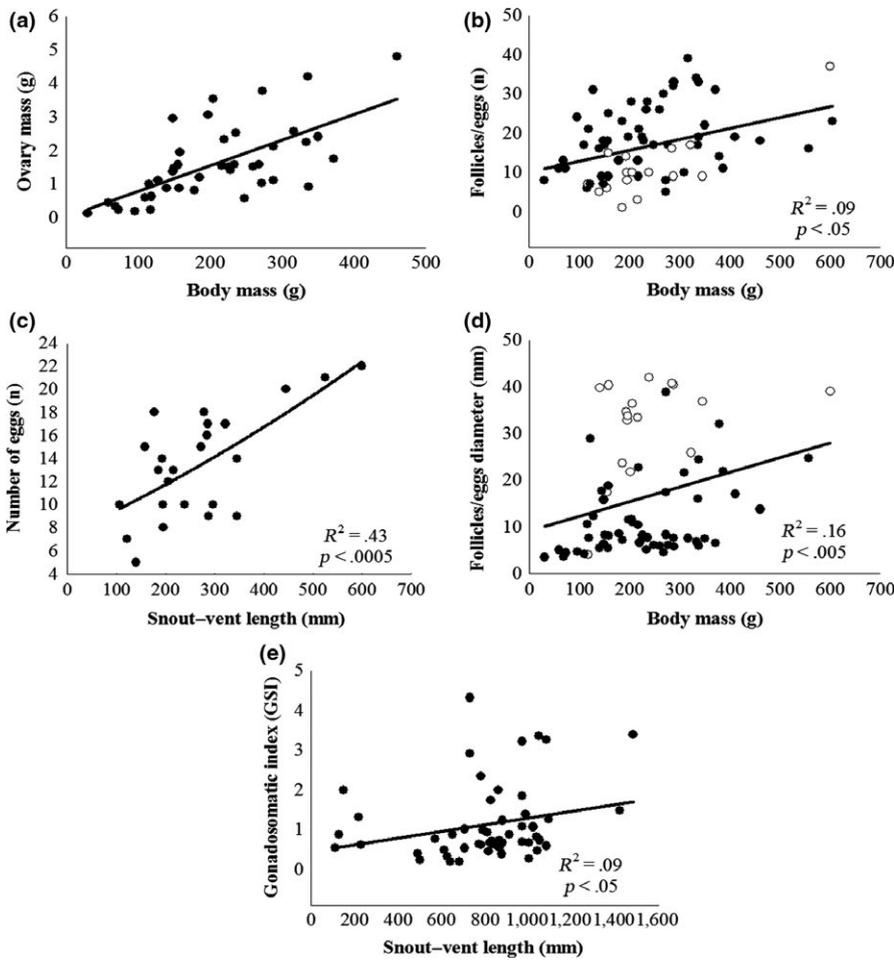


FIGURE 4 Relationship between (a) ovary mass and body mass, (b) number of follicles/eggs and body mass, (c) clutch size and SVL for females, (d) follicles/eggs diameter and body mass and (e) gonadosomatic index and body size of *Philodryas patagoniensis* in south Brazil. (●): vitellogenic follicles. (○): eggs

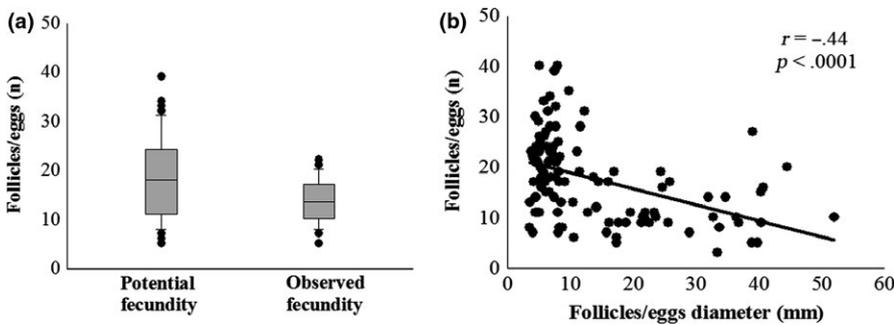


FIGURE 5 (a) Potential vs. observed fecundity; (b) relationship between follicles/eggs diameter (size) and follicles/eggs number of *Philodryas patagoniensis* in south Brazil. Middle line represents average values, boxes show standard deviations, and whiskers represent minimum and maximum values

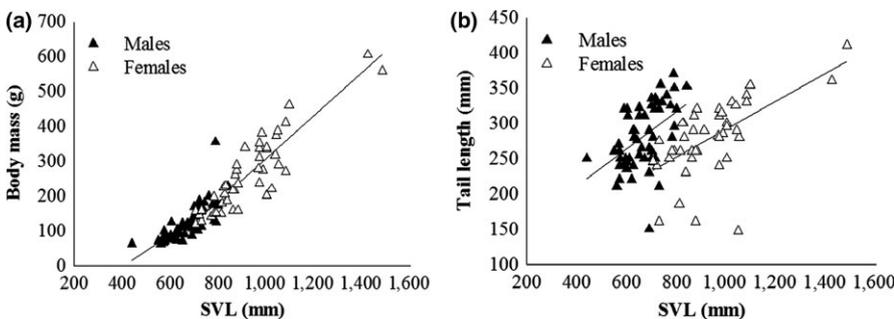


FIGURE 6 Sexual dimorphism in (a) body size and (b) tail length of *Philodryas patagoniensis* in south Brazil. (▲): males. (△): females

(2011). At the individual level, females show a discontinuous cyclical reproduction, while in the population level, the reproductive cycle is seasonal semisynchronous with most of

the individuals showing a reproduction peak in spring–summer. In south-eastern Brazil (Espírito Santo, Minas Gerais, Rio de Janeiro and São Paulo States), *P. patagoniensis* also

presents a seasonal pattern of reproduction, with secondary vitellogenesis occurring from winter to spring, ovulation in spring, oviposition in spring–summer and newborns hatching in summer (Fowler et al., 1998; Rojas, 2013). The values of GSI, follicle/eggs diameter and shell (uterine) glands diameter increased during winter–spring, coinciding with the reproductive season. Females, which had secondary follicles in late developmental stage, presented hypertrophied uterine glands full of secretory granules. A recent histochemical study of *P. pataginiensis* oviduct revealed hypertrophy of the uterine glands and increasing production of secretory granules during the secondary vitellogenesis (Rojas et al., 2015). The uterine glands have an important role in the development of eggs, providing the pseudokeratin, which is a component of egg shell membranes (Perkins & Palmer, 1996).

The ability to store sperm for more than one season might be related to sperm-storage structures identified in caudal (UVJ) and cranial sites (posterior infundibulum) of *P. patagoniensis* oviduct. Females had sperm groups stored in furrows of the UVJ during all the seasons, but in higher frequency from winter to summer (July–March). However, the occurrence of sperm storage in the posterior infundibulum was deduced merely by the occurrence of sperm-storage receptacles with sperm debris from spring to summer (October–March). Similar results were found in *P. patagoniensis* from south-eastern Brazil when sperm-storage patterns were analysed in Xenodontinae (Rojas, 2013). However, in *P. patagoniensis* from that region, sperm storage was clearly evidenced in the posterior infundibulum and in the UVJ (Rojas et al., 2015). Therefore, we conclude that long-term sperm storage takes place in deep furrows (crypts) of the UVJ, while short-term sperm storage occurs in the infundibulum receptacles. In species with infundibular storage, sperm is first stored in the UVJ for some period until cranial migration to infundibulum receptacles just prior fertilization (Almeida-Santos & Salomão, 1997; Fox, 1956; Hoffman & Wimsatt, 1972; Perkins & Palmer, 1996). Sperm-storage structures in the UVJ have been described as highly unspecialized and not differentiated glands from the oviduct epithelium (Sever & Ryan, 1999) that physically protect the sperm from getting caudally swept in the oviduct by the eggs (Aldridge, 1992).

The fertilization success of stored sperm by the time of ovulation was not clearly assessed; however, it is known that sperm can reside in the UVJ for at least a few weeks up to over the hibernation period (Siegel, Miralles, Chabarria, & Aldridge, 2011). In *Thamnophis sirtalis* uterus, the sperm could survive for at least 1 month (Rahn, 1940). The presence of sperm stored in the UVJ of pregnant and recently oviposited females suggests that *P. patagoniensis* does not eliminate the sperm after ovulation and can use it to produce several clutches (Rojas et al., 2015). The presence or absence of multiple clutches is considered one of the most fascinating traits that are expected to vary among the squamate (Ford

& Siegel, 2006). The evidence of follicles and eggs simultaneously mature suggests that *P. patagoniensis* should be able to produce multiple clutches during the same reproductive season (Shine, 1977). This reproductive advantage is allowed due to the ability to store sperm in the UVJ. The production of at least two consecutive clutches is now well documented for a number of snakes from Brazilian tropical region (Marques, 1996; Marques & Muriel, 2007; Mesquita, Borges-Nojosa, Passos, & Bezerra, 2011; Pinto & Fernandes, 2004; Pinto, Marques, & Fernandes, 2010; Vitt, 1983) and subtropical region (Aguir & Di-Bernardo, 2005; Balestrin & Di-Bernardo, 2005; Mesquita, Sá-Polidoro, & Cechin, 2013). *P. patagoniensis* produces multiple clutches per season and, due to this characteristic, has extended reproduction.

Sexual dimorphism in body size has been previously reported to *P. patagoniensis* (Fowler & Salomão, 1994; Hartmann & Marques, 2005; López & Giraud, 2008); females are reported to be larger than males which is a common pattern in snakes (Shine, 1988). The reproduction-related processes that act on the organisms' body size may be distinguished between fecundity selection and sexual selection (Bonnet, Naulleau, Shine, & Lourdis, 2000). Body size sexual dimorphism may be correlated to size selection based on fecundity because larger females produce larger clutches (Shine, 1994). Late sexual maturity is advantageous in a way that it allows females to reach larger body sizes before starting reproduction events. Furthermore, the sexual selection may favour larger females with better body condition because males are able to distinguish information about body condition, age, reproductive state and species based on females' skin lipid pheromones (LeMaster & Mason, 2002; Shine, Phillips, Waye, LeMaster, & Mason, 2003). To the majority of snake species, a female-biased SSD is the rule, while the sexual selection can influence this trait in lineages that exhibit pre-copulatory male–male interactions (Shine et al., 2000). Therefore, a female-biased SSD is considered an ancestral condition in snakes (Rivas & Burghardt, 2000). On the other hand, patterns of female-biased SSD are consistent with the hypothesis that sexual selection favours small-sized males, which are more efficient in obtaining coercive mating (Shine & Mason, 2005).

Larger females of *P. patagoniensis* produce follicles and eggs in larger amount and size. The maternal body size is directly related to the fecundity, and larger females produce larger clutches than their smaller conspecifics (Shine, 1994). This can be explained because larger females have larger abdominal volume to accommodate the eggs (Brown & Shine, 2005). Snakes offspring are mother-size-dependent, but in *P. patagoniensis*, both fecundity and clutch size widely vary, indicating that are many other factors involved in the reproductive investment (Ford & Siegel, 2011). However, it is difficult to determine the degree of offspring variation influenced by the genotype and/or the environment.

TABLE 2 Phases of the annual reproductive cycle of *Philodryas patagoniensis* females in south Brazil

	Summer	Autumn	Winter	Spring
Peak activity of males and females				■
Peak activity of juveniles	■			
Secondary vitellogenesis peak			■	■
GSI increases			■	■
Shell glands hypertrophy			■	■
Long-term sperm storage (utero-vaginal junction)	■	■		
Short-term sperm storage (infundibulum)	■	■		
Ovulation	■	■		
Mating	■	■	■	
Gravidity	■	■	■	
Presence of follicles and eggs			■	■
Oviposition			■	■
Births/hatchling			■	■

Shades area correspond to the season of activity.

Philodryas patagoniensis exhibits a higher potential fecundity (secondary follicles) compared with the realized/observed fecundity (eggs). Vitellogenesis may represent the major reproductive investment in females and producing more follicles/eggs results in a reduction of follicles/eggs size. This may occur because females are able to control the determination of an optimal clutch size-number that will provide a higher maternal fitness in view of the limited energy store or abdominal volume available for the clutch (Brown & Shine, 2009). However, the trade-off between reproductive investment and maternal fitness presupposes that an increase in the reproductive investment negatively affects as other components of fitness such as the female survival, growth rates and the future reproductive output (Brown & Weatherhead, 2004; Shine & Schwarzkopf, 1992). Another related trade-off is between maternal fitness and offspring fitness, because it is expected that larger neonates should have several survival advantages: increased locomotion ability to escape predators, shorter time to sexual maturity and enhanced foraging success (Hernandez-Salinas & Ramirez-Bautista, 2014; Kissner & Weatherhead, 2005). However, in oviparous snakes, the neonate size (SVL and/or mass) can be influenced not only by the initial egg mass, but also by the incubation period and egg mass gained during incubation (Brown & Shine, 2005).

In Table 2, we summarized the principal events of the *P. patagoniensis* female reproductive cycle in south Brazil, considering the synchrony with the male cycle (Loebens et al., 2016). Both, males and females exhibit reproductive cycle of the type seasonal semisynchronous, with a strong peak in spring–summer. Both sexes present strategies of sperm storage, maximizing the reproductive success. The males are able to provide viable sperm during early spring matings, and the female can store sperm for a considerable time after the mating. The females do not eliminate the sperm after ovulation, and this allow the production of more than one clutch. Therefore, *P. patagoniensis* have an extended seasonal pattern of reproduction in subtropical Brazil. Meanwhile, some reproductive aspects such as sperm storage and production of multiple clutches require more attention, mainly when analysed through a phylogenetic perspective. To completely understand the species reproductive traits and patterns, more field and experimental studies are required to clarify questions about sex steroids and environmental cues, for example. Although many further opportunities are still available, the knowledge about subtropical squamates reproduction should be enlarged with this study.

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APPENDIX

Voucher specimens of *Philodryas patagoniensis* analysed in this study housed in the herpetological collections of the Federal University of Rio Grande do Sul (UFRGS), Federal University of Santa Maria (ZUFMS) and Pontifical Catholic University of Rio Grande do Sul (MCP-PUCRS)

Macroscopic and anatomical analysis ($n = 112$): ZUFMS0118, 0146, 0261, 0262, 0331, 0342, 0360, 0388, 0399, 0433, 0550, 0652, 0666, 0681, 0684, 0708, 0723, 0866, 0967, 0971, 1088, 1089, 1109, 1130, 1144, 1156, 1171, 1295, 1398, 1404, 1405, 1441, 1650, 1744, 1801, 1855, 1935, 2056, 2089, 2169, 2336, 2431, 2451, 2452, 2476, 2504, 2538, 2581, 2640, 2670, 2696, 2698, 2718, 2720, 2761, 2771, 2986, 3107 and 3112. MCP05257, 05774, 06059, 06158, 12712, 12782, 12793, 12794, 13184, 13185, 13305, 13306, 13411, 13894, 14118, 14325, 14513, 14567, 14568, 14650, 14858, 14995, 15732, 15924, 16144, 16960, 16965, 16966, 16968, 16972, 16978, 16979, 16980, 16981, 16982, 16984, 17013, 17014, 17015, 17016, 17017, 17019, 17021, 17022, 17795, 17837, 17858, 17972, 18133, 18286, 18858 and 19054.

Histology ($n = 55$): ZUFMS0118, 0146, 0262, 0331, 0342, 0360, 0388, 0399, 0433, 0666, 0708, 0866, 0967, 0971, 1088, 1089, 1109, 1130, 1144, 1156, 1295, 1398, 1404, 1441, 1650, 1744, 1801, 1855, 2056, 2431, 2476, 2581, 2718, 2761, 2771 and 3107. MCP12793, 12794, 14567, 16960, 16966, 16968, 16972, 16979, 16981, 16982, 17013, 17016, 17017, 17021, 17022, 17837, 18286, 18858 and 19054.