ADJUSTMENT OF THE REPRODUCTIVE ACTIVITY OF VULNERABLE LIZARD *PHYMATURUS WILLIAMSI* AT HIGH ALTITUDES

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Abstract.—In environments with cold climates, reproduction of lizards is restricted to short seasonal periods of activity, from mid-spring to early autumn. We studied the reproductive biology of *Phymaturus williamsi* (William's Andean Lizard), a vulnerable and viviparous lizard from the *palluma* phylogenetic group of genus *Phymaturus*, endemic to rocky outcrops in cold environments in the Andes of San Juan, Argentina. We determined adult size, male and female reproductive cycles, litter size, mean annual reproductive output, and the relationship between gonadal cycles, fat bodies, and climatic variables. The shortest reproductive male and female were 87.2 mm and 86.4 mm SVL, respectively. The litter size per female was of one or two offspring every two years, and the mean annual reproductive output was 0.95 offspring/female/year. Births occurred from late summer to early autumn. Males exhibited an annual postnuptial cycle, with spermatogenesis beginning in mid-spring, spermiogenesis occurring in summer and autumn, and sperm stored during brumation through early spring when mating occurs. The male post-nuptial cycle is common in species of the *patagonicus* group, but not in the *palluma* group, as is *P. williamsi*. Nevertheless, the biennial female cycle is common in the *palluma* group, and results from the adjustment of the reproductive activity to the physiological and environmental constraints of the harsh and cold climatic conditions prevailing at high altitudes in the Andes. The knowledge of the reproductive traits of the vulnerable *P. williamsi* has direct relevance for the development of conservation strategies, as this information is fundamental for population viability analyses.

Key Words.—biennial reproduction; environmental constraints; harsh environments; physiological constraints; viviparity

INTRODUCTION

In ectotherms, the timing of reproductive activity is widely variable and influenced by the environment (Dubey and Shine 2011) and by their phylogeny (Watson et al. 2014). In environments with cold and harsh climatic conditions, lizards have long periods of brumation and short seasonal periods of activity, being under physiological and environmental constraints for reproduction (Saint Girons 1985; Andrews and Mathies 2000; Boretto et al. 2014a,b). Particularly, in viviparous lizard species, high levels of progesterone during gestation inhibits follicular development and vitellogenesis, limiting the female reproductive cycle due to the physiological inability of females to perform vitellogenesis while gravid (Duvall et al. 1982; Callard et al. 1992; Custodia-Lora and Callard 2002). As consequence of these constraints, viviparous females that inhabit cold environments may display prolonged reproductive cycles producing litters every two (biennial), or even three (triennial) years (Ibargüengoytía and Cussac 1999; Boretto and Ibargüengoytía 2006;

Boretto and Ibargüengoytía 2009). Under this scenario, species with low litter size and a prolonged female reproductive cycle exhibit a low reproductive rate and, in consequence, a low mean annual reproductive output (Cree 1994; Boretto et al. 2007; Boretto and Ibargüengoytía 2009; Boretto et al. 2014b). These life-history parameters determine the number of reproductive events during the life of an individual and, consequently, the cumulative cost of reproduction (Cree 1994), information that is of crucial importance for the development of species-specific conservation strategies.

We studied the reproductive biology of *Phymaturus williamsi*, an endemic lizard to rocky outcrops in the highlands of the Central Andes of Argentina. All species of the genus *Phymaturus* (Liolaemidae) are viviparous, saxicolous, and mostly herbivorous, and are distributed in cold environments in northwest Argentina from the Catamarca Province along the Andes mountain range and adjacent areas in Chile to the Patagonian steppe of Argentina (Cei 1993). *Phymaturus* includes two large clades based on morphological traits (Cei 1986; Etheridge 1995) and mitochondrial sequence evidence

FIGURE 1. Adults male (left) and female (right) of William´s Andean Lizard (*Phymaturus williamsi*) from near Quebrada de La Puerta, Calingasta, San Juan, Argentina. (Photographed by Ana Victorica).

(Espinoza et al. 2004; Morando et al. 2013): the *palluma* group, in which *P. williamsi* is included (*sensu* Cei 1993; Cei and Scolaro 2006), and the *patagonicus* group (Cei 1993; Lobo et al. 2013). Recently, the number of described species of the genus *Phymaturus* has grown significantly (Lobo et al. 2013; Lobo and Nenda 2015; Marín et al. 2016). However, reproductive biology remains unknown for most species. Detailed studies of the reproductive cycles and, in particular, the times of each reproductive event in males and females, the frequency of female reproduction, litter size, body sizes at sexual maturity, and male reproductive strategy are relevant to conservation, as this information has a direct impact on the construction of population viability analyses (Stearns 2000; Boretto et al. 2017). In this sense, the study of the reproductive biology in *Phymaturus* species is of special interest as the entire genus has been categorized as vulnerable due to significant microendemisms of numerous species (Abdala et al. 2012), their specialist saxicolous habits (Cei 1986), mostly herbivorous diet (Cei 1986; Boretto and Ibargüengoytía 2009; Castro et al. 2013), viviparous reproductive mode (Cei 1986), and low annual reproductive output (Boretto and Ibargüengoytía 2006, 2009; Boretto et al. 2007, 2014b; Cabezas-Cartes et al. 2010).

We studied the reproductive cycle of males and females of *P. williamsi* and determined minimum adult size, litter size, and mean annual reproductive output. We also analyzed the relationship between gonadal cycles, fat body cycles, and climatic variables to relate the reproductive biology to the energetic, physiological, and environmental constraints the species faces. Finally, to discuss our results in a broader context, we compared the reproductive biology of *P. williamsi* with other viviparous lizards with prolonged female reproductive cycles that inhabit temperate environments.

MATERIALS AND METHODS

Study site and specimens.—We collected 40 male, 54 female, and 13 newborn *Phymaturus williamsi* (Fig. 1) during spring (October-November 2009–2010), summer (December 2009–2010, March 2010) and autumn (April 2011) in the locality Quebrada de La Puerta, Calingasta, San Juan, Argentina (32°31.31'S; 69°42.02'W; 2,878 m above sea level). The study area belongs to the phytogeographic province of the Puna (Cabrera and Willink 1973), in the Andean highlands of Argentina, on the west bank of the Iglesia-Calingasta-Uspallata Valley, with a north-south orientation. We obtained the mean monthly temperature (° C) and rainfall (mm) data for September 2009 to April 2011 from meteorological stations (Troy Resources Argentina, Ltd.) located 20 km from the study area.

We captured lizards by hand or by noose (slipknot), and we determined the sex (using the presence of precloacal pores in males) and measured the snout-vent length (SVL) using digital dial calipers (± 0.2 mm). We brought these lizards to the laboratory and euthanized them by intraperitoneal administration of sodium thiopental anesthetics fixed in Bouin's solution for 24 h. We preserved each specimen in 70% ethanol. We excised and weighed the fat bodies of each specimen

with an analytical balance (± 0.0001 g, AGZN200, Torbal, Poland). We deposited the specimens in the Scientific Herpetological Collection of the Facultad de Ciencias Exactas, Físicas y Naturales at the Universidad Nacional de San Juan, Argentina.

Female reproductive traits.—We determined the minimum SVL at sexual maturity based on the smallest female with vitellogenic follicles in the ovary (vitellogenic female), embryos in the uterus (pregnant female), or a distended uterus characteristic of postpartum females or adult non-reproductive females (detailed description below). We measured the follicular sizes, estimated as the diameter of the largest ovarian follicle, with digital dial calipers (± 0.2 mm). We determined the litter size by counting the total number of the embryos present in the right and left uterus of each female. Then we calculated the mean annual reproductive output as the mean number of offspring per female per year (*sensu* Cree 1994). We classify the embryos into one of three stages of development (*sensu* Leyton et al. 1980): early (from cleavage to somitic embryos), medium (curvate trunk to limbs with five fused fingers, outlines of ears and eyes, and abundant yolk), and advanced (scales and pigmented skin). Based on the characteristics of the reproductive tract and the follicular development (Boretto et al. 2014b), we classified females as juvenile (uteri like a thread, transparent, without folds, and with previtellogenic and small follicles), adult vitellogenic (distended uteri with medium-size folds over the entire surface, with enlarged vitellogenic follicles), non-reproductive adult (distended uteri with medium size folds and small vitellogenic follicles), or postpartum adult female (conspicuous and expanded uteri, with numerous and large folds over the entire surface, and the presence of corpus luteum, indicative of recent parturition).

Male reproductive traits.—We determined the minimum SVL at sexual maturity based on the smallest male showing spermatogenesis. Arbitrarily, we removed the right testis and epididymis of each specimen and dehydrated them in an ethanol series and embedded in paraffin. We stained the sections (7 mm) with Masson trichromic and we examined them under a stereoscopic microscope (BX40, Olympus America Inc., New York, New York, USA) equipped with a Pro-Series High-Performance CCD Camera, and an Image-Pro Plus analyzer (Media Cybernetics, Inc., Rockville, Maryland, USA). We determined the spermatogenic stages following Mayhew and Wright (1970) based on the most advanced cell type presented at the luminal margin of the seminiferous tubule, as: (1) only spermatogonia, (2) primary and secondary spermatocytes, (3) spermatids, (4) spermatozoa in the tubular lumen and epididymis,

and (5) testicular regression evidenced by the presence of cellular debris in lumen.

Statistical analyses.—We used Sigma Stat 3.5® (Systat Software, Inc., Chicago, Illinois, USA), Sigma Plot 10.0® (Systat Software, Inc., Chicago, Illinois, USA) and SPSS 17.0® (SPSS, Inc., Chicago, Illinois, USA) to analyze data. We tested assumptions of normality and homogeneity of variance with the one-sample Kolmogorov-Smirnov test and with the Levene test, respectively. We transformed the data corresponding to sampling date into numerical data because we previously modified the years of capture to treat the data as corresponding to a single year. For the analysis of the relationship between quantitative and continuous variables we used Linear Regression. If the assumptions of normality and homogeneity of variance were not met, we used Spearman correlation. For the analysis of the relationship between quantitative and categorical variables, we used Spearman correlation. For example, in the analysis of the relationship between SVL and follicular size, we used Spearman correlation because the data were heteroscedastic, while for the analysis of the relationship between SVL and testicular size, the data were homoscedastic and we used Linear Regression.

Because testicular size of adult males was positively related to SVL, we used the residuals of the linear regression (ResTS) of testicular size related to SVL for the rest of the analyses. For the relationship between monthly highest follicular or testicular size or monthly proportion of males with sperm, with ambient temperature or rainfall, we used Multiple Regression. To analyze the relationship between fat body weight and SVL or date, we used Linear Regressions, and for the relationship between fat body weight and spermatogenic stage we used Spearman correlation. We used ANOVA to analyze the differences in fat body weights between adult females and adult males with different reproductive conditions, and then we used *posteriori* tests Holm-Sidak or Dunn's Method to find pair-wise differences. We used Kruskal-Wallis test when the data were not normal or homoscedastic, which was the case for the differences in fat body weight between males and females and between males with the absence or the presence of abundant or scarce sperm in the epididymis. Means are given with ± 1 standard error (SE), and for tests, α = 0.05.

RESULTS

The minimum adult size in a vitellogenic female was 86.4 mm SVL, and 87.2 mm SVL in a male with the testicular regression stage. For 42 sexually mature females, SVL ranged from 86.4 to 104.6 mm (mean =
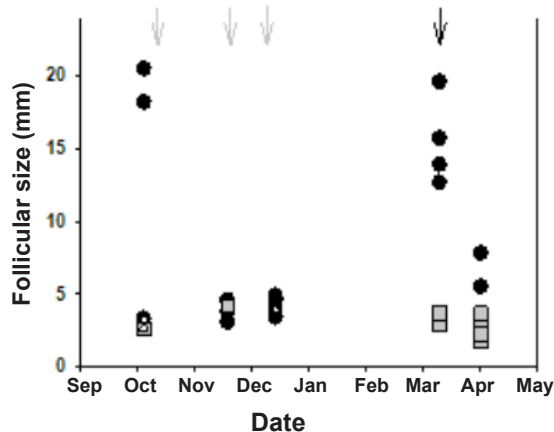
FIGURE 2. Follicular sizes of non-reproductive (triangles), vitellogenic (circles) and postpartum (square) female William´s Andean Lizard (*Phymaturus williamsi*) from near Quebrada de La Puerta, Calingasta, San Juan, Argentina, in relation to capture date. Arrows indicate the capture date of pregnant females with embryos in early (grey) and advanced (black) developmental stages.

FIGURE 3. Reproductive body size (follicle size in mm, grey circles; testicle size in mm, white squares) in adult William´s Andean Lizard (*Phymaturus williamsi*) from near Quebrada de La Puerta, Calingasta, San Juan, Argentina, by capture date, from September 2009 to September 2010 in relation to monthly mean temperature (°C, solid line) and rainfall (mm, dashed line).

97.3 ± (SE) 0.66 mm), and from 87.2 to 106.2 mm in 40 adult males (mean = 97.8 ± 0.71 mm). In juveniles, SVL ranged from 64.9 mm to 88.0 mm in 10 females (mean = 80.0 ± 2.20 mm), and from 80.0 mm to 82.0 mm SVL in two males. Thirteen specimens with SVL of 53.2 mm to 64.4 mm (mean = 60.7 ± 0.99 mm) had umbilicus scars and we assumed them to be newborns.

Female reproductive cycle and environmental variables.—Follicular size (FS) of vitellogenic females was positively related to date (n = 19, r = 0.66, P = 0.002), indicating that vitellogenesis began from November (highest FS = 4.6 mm) to March (highest FS = 19.6 mm), reaching a peak after brumation in October of the next year (FS = 20.5 mm), extending for approximately 11 mo (Fig. 2). The monthly highest follicular size was not related to the monthly mean temperature nor the rainfall ($F_{2,6}$ = 0.51, P = 0.635).

Throughout the activity season, we simultaneously captured juvenile females (n_{spring} = 6, FS_{range} = 1.8–3.0 mm; nearly summer = 1, FS = 3.0 mm; $n_{late summer}$ = 5, FS = 1.5–2.4 mm), vitellogenic females (n_{spring} = 6; n_{summer} = 11; $n_{early autumn}$ = 2), non-reproductive females (n_{spring} = 4; n_{summer} = 1), and pregnant females (n_{spring} = 5; $n_{early summer}$ = 4; $n_{late summer}$ =1). We found postpartum females in late summer (n = 2), early autumn (n = 4), and spring (n = 2). We observed newborns in spring (n = 7, SVL_{mean} = 60.0 mm ± 1.59 mm), early summer (n = 2, SVL_{mean} = 61.4 mm ± 2.40 mm), and late summer (n = 4, SVL_{mean} = 61.4 mm ± 1.60 mm). All pregnant females exhibited two embryos each (one embryo per uterus), with the exception of one female that presented only one embryo. From early spring to late summer, we captured pregnant females with embryos with early development, whereas
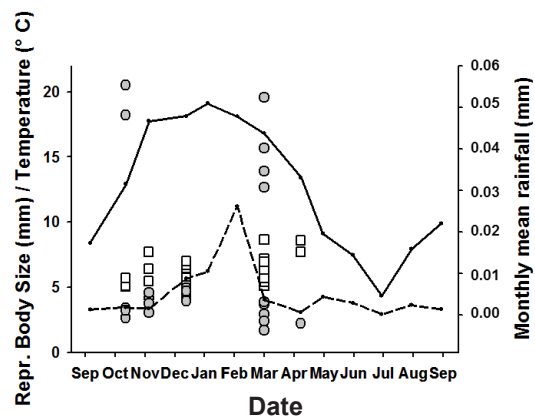
in late summer we captured a female with embryos with advanced development (Fig. 2). Considering that mean clutch size was 1.9, and the biennial reproductive cycle (annual 1; biennial = 0.5), we estimated a mean annual reproductive output of 0.95 offspring/female/year (1.9 × 1 × 0.5 = 0.95; *sensu* Cree 1994).

Male reproductive cycle and environmental variables.—Testicular size of adult males was positively related to SVL ($F_{1,39}$ = 28.06, P < 0.001). Consequently, we used the residuals of the linear regression (ResTS) of testicular size related to SVL for further analyses. The ResTS were not related to the spermatogenic stages (n = 40, r = 0.09, P = 0.562). In addition, monthly highest testicular size was not related to the monthly mean temperature nor the rainfall ($F_{2,6}$ = 0.73, P = 0.535). Also, the monthly proportion of males with sperm were not related to the monthly mean temperature or the rainfall ($F_{2,6}$ = 0.40, P = 0.692; Fig. 3).

The spermatogenic cycle of *P. williamsi* started in mid-spring, after the brumation period, when males exhibited spermatids with scarce or absent sperm in the epididymis (n = 2; stage 3; October 2009–2010, November 2010). In this season, there were also found males with testicular regression (n = 4; stage 5) and some exhibited scarce sperm in the epididymis (80% of males; Fig. 4). In early summer, we captured males with different spermatogenic stages in testis (n = 2, stage 2; n = 2, stage 3; n = 6, stage 4; December 2009–2010), and half of these males with spermiogenesis (three males with stage 4) and one male with stage 2 showed scarce sperm in the epididymis. In late summer, males showed spermiogenesis (n = 6; stage 4) with abundant sperm in the epididymis (83% of males), or testicular regression
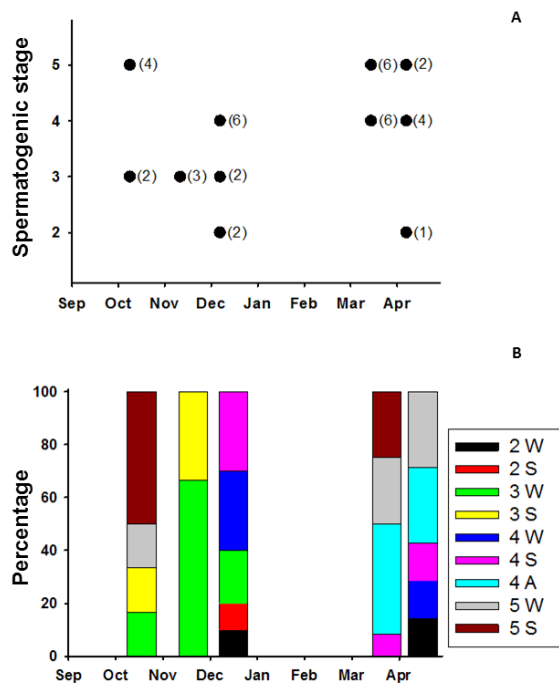
FIGURE 4. Spermatogenic stages of male William´s Andean Lizard (*Phymaturus williamsi*) from near Quebrada de La Puerta, Calingasta, San Juan, Argentina, in relation to capture date. The number of individuals for each data point is in parentheses next to the data point. (Top) Spermatogenic stage: (2) primary and secondary spermatocytes; (3) spermatids; (4) spermatozoa in tubular lumen; and (5) regression. (Bottom) Percentages of males with abundant (A: black), scarce (S: grey), or without spermatozoa in the epididymis (W: white).

(n = 6, stage 5) with scarce sperm in the epididymis (50% of males; March 2011; Fig. 4). In early autumn, we captured males with spermiogenesis (n = 4; stage 4) with abundant sperm in the epididymis (50% males) or testicular regression without sperm in the epididymis (n = 2; stage 5; April 2010).

Fat storage.—Fat body weight of adult females was not related to SVL (n = 42, $P = 0.706$), and fat body weight of non-pregnant females (non-reproductive, vitellogenic and postpartum) was not related to SVL (n = 32, $F_{1,31} < 0.001$, $P = 0.988$) or capture date (n = 32, $F_{1,31} < 0.001$, $P = 0.675$). Vitellogenic, pregnant, and postpartum females showed significant differences in fat body weight ($F_{2,36} = 4.64$, $P = 0.016$), since vitellogenic females showed higher fat body weight than pregnant females (Holm-Sidak, $t = 2.96$, $P = 0.017$; mean$_{vitellogenic}$ = 0.46 ± 0.24 g, n = 19; mean$_{pregnant}$ = 0.20 ± 0.14 g, n = 10). Vitellogenic, postpartum and non-reproductive females did not show significant differences in fat body weight ($F_{2,31} = 1.26$, $P = 0.299$).

Fat body weight of adult males was not related to SVL (n = 38, $P = 0.737$), spermatogenic stage (n = 38, $P = 0.380$), or capture date (n = 38, $F_{1,37} = 2.82$, $P = 0.102$).

Fat body weight was not different between males with abundant, scarce, or without sperm in the epididymis ($H = 1.21$, df = 2, $P = 0.547$). Fat body weight of adult reproductive individuals (males, vitellogenic and pregnant plus post-partum females) showed significant differences ($H = 15.92$, df = 2, $P < 0.001$), with vitellogenic females having significantly higher fat body weight than males (Dunn's Method, $Q = 3.95$, $P < 0.05$) and pregnant plus post-partum females ($Q = 2.83$, $P < 0.05$). Males and pregnant plus post-partum females did not show significant differences in fat body weight ($Q = 0.88$, $P > 0.05$).

DISCUSSION

In the cold environments of the highlands of the Andes, *Phymaturus williamsi* have periods of annual activity restricted to early spring until early autumn, during which we observed the simultaneous presence of vitellogenic females and pregnant females with early to advanced embryonic development, indicating that *P. williamsi* has a biennial female reproductive cycle. Females of *P. williamsi* require one activity season to complete the vitellogenic process (extended for 11 mo) and then ovulate, copulate and start the gestation period, which requires at least 6 mo. As such, each female reproductive cycle required two years for completion, considering the brumation period after gestation and births. Thus, biennial female reproductive cycles seem to be characteristic of the *palluma* phylogenetic clade to which *P. williamsi* belongs, in which all species inhabit cold environments at high altitudes in the Andes (*P. vociferator*, Habit and Ortiz 1996; *P. antofagastensis*, Boretto and Ibargüengoytía 2006; *P. punae*, Boretto et al. 2007; *P. aguanegra*, Cabezas-Cartes et al. 2010). Biennial female cycles were also found in some viviparous species endemic to New Zealand, Australia, South Africa, and Chile (Table 1). However, in the *patagonicus* clade of genus *Phymaturus*, species inhabits low altitudes in the Patagonian steppe, and females can reproduce biennially (*P. tenebrosus*, Ibargüengoytía 2004) or annually/biennially (*P. zapalensis*, Boretto and Ibargüengoytía 2009; *P. spectabilis*, Boretto et al. 2014b). The flexibility in frequency of female reproduction could be a coping mechanism for unpredictable climates or when the thermal quality and length of the activity season vary (Edwards et al. 2002). For example, females of *P. spectabilis* in the Patagonian steppe clearly skipped reproduction for one year as a mechanism for coping with the detrimental environmental changes caused by the eruption of the Puyehue Volcano (Boretto et al. 2014a). Also, viviparous *Liolaemus* from the Patagonian Highlands can interrupt their reproductive activity for one or two years (Ibargüengoytía and Cussac 1996, 1998), similar

TABLE 1. Location, elevation, long reproductive event, mean clutch size, and mean annual reproductive output (offspring/female/year) of viviparous lizards with prolonged reproductive cycles. Abbreviations are LRE = long reproductive event, MCS = mean clutch size, MARO = mean annual reproductive output, and asl = above sea level. One asterisk (*) is mean annual reproductive output calculated in Ibargüengoytía and Casalins 2007; two asterisks (**) is mean annual reproductive output calculated using the mean clutch sizes cited by authors.

Species	Location, elevation	LRE	MCS	MARO	Reference
New Zealand					
Hoplodactylus maculatus	Region Central Otago; 300–700 m asl	gestation	2	0.85	Cree and Guillette 1995
Naultinus gemmeus	Península Otago; 40–90 m asl	gestation	2		Wilson and Cree 2003
Australia					
Niveoscincus microlepidotus	Tasmania	gestation	2.3	1.38	Olsson and Shine 1999, Cree 1994
Tiliqua nigrolutea	Southeastern Australia	—	8.3	2.97*	Edwards et al. 2002
South Africa					
Cordylus giganteus	Highveld grasslands; 1,620 m asl	vitellogenesis	2.7	1.12*	Van Wyk 1991
Chile					
Phymaturus vociferator	Parque Nacional Laguna del Laja, Chile; 1,700 m asl	gestation	1.5	0.75**	Habit and Ortiz 1996
Argentina					
Phymaturus antofagastensis	Paso Internacional San Francisco, Catamarca, 4,200 m asl	vitellogenesis	2	1*	Boretto and Ibargüengoytía 2006
Phymaturus punae	Reserva Provincial San Guillermo, San Juan; 3,100–4,200 m asl	vitellogenesis	2	0.75	Boretto et al. 2007
Phymaturus aguanegra	Agua Negra, Iglesia, San Juan; 2,900 m asl	vitellogenesis	2	0.75	Cabezas-Cartes et al. 2010
Phymaturus tenebrosus	Río Negro y Neuquén; 575–1,230 m asl	vitellogenesis	2	1*	Ibargüengoytía 2004
Phymaturus zapalensis	Parque Nacional Laguna Blanca, Zapala, Neuquén; 824–1,312 m asl	skipping year	1.5	1.12	Boretto and Ibargüengoytía 2009
Phymaturus spectabilis	Ingeniero Jacobacci, Río Negro; 983-1064 m asl	skipping year	2	0.75	Boretto et al. 2014a,b
Liolaemus pictus	Parque Nacional Nahuel Huapi y Lanin; 500–1,800 m asl	vitellogenesis	4.5*	1.87*	Ibargüengoytía and Cussac 1996
Liolaemus elongatus	Same as above	vitellogenesis	4.66*	3.49*	Ibargüengoytía and Cussac 1998
Liolaemus magellanicus	Santa Cruz; 133 m asl	vitellogenesis	3.5	2.55	Fernández et al. 2015
Liolaemus sarmientoi	Same as above	vitellogenesis	4.5	3.09	Fernández et al. 2015

to the viviparous scincid *Tiliqua nigrolutea* from cold environments in Tasmania (Edwards et al. 2002; Table 1). Instead, we did not observe flexibility or plasticity in the reproductive frequency of *P. williamsi* under natural conditions over two years; the female cycle is strictly biennial.

The energetic cost of each reproductive stage of the female reproductive cycle seems to drive the differences in the time allocated for each. In species with biennial cycles, females can allocate short periods to vitellogenesis and long periods to gestation, as occurs in *P. vociferator* (Habit and Ortiz 1996) and in viviparous species that inhabit similar latitudes in New Zealand (*Hoplodactylus maculatus*, Cree and Guillette 1995; *Naultinus gemmeus*, Wilson and Cree 2003), and Australia (*Niveoscincus microlepidotus*, Olsson and Shine 1999), but at altitudes lower than 1,500 m (Table 1); or they can allocate long periods to vitellogenesis and short periods to gestation, as in *P. williamsi* (present study) and in others *Phymaturus* (Table 1). Prolonged vitellogenesis has also been described in viviparous *Liolaemus* from the Argentinean Patagonia (*L. elongatus*, Ibargüengoytía and Cussac 1998; *L. magellanicus*, and *L. sarmientoi*, Fernández et al. 2015), and in species endemic to Highveld Grasslands in South Africa (*Cordylus giganteus*, Van Wyk 1991; Table 1). Vitellogenesis appears to be more expensive than pregnancy, because yolk energy content is the most

significant component of the reptilian reproductive effort and has a higher metabolic cost than pregnancy, at least in lecithotrophic viviparous species (Van Dyke and Beaupre 2011) like *Phymaturus* (Boretto et al. 2015).

Despite the different times allotted to each reproductive stage, births occurred from mid- to late summer in all *Phymaturus* studied (Boretto and Ibargüengoytía 2009; Cabezas-Cartes et al. 2010; Boretto et al. 2014a,b). However, we found that in *P. williamsi* births occurred from late summer to early autumn, later than other *Phymaturus*, based on the presence of pregnant females with an advanced stage of embryonic development in late summer and postpartum females from late summer to early autumn. The body size of newborns captured in spring and early summer indicated that these individuals may have been born during the last autumn. Our study confirmed a litter size of one to two offspring per female in *P. williamsi*, and consequently, a low mean annual reproductive output of 0.95 offspring/female/year, a value close to the lowest recorded outputs for lizards worldwide (Cree 1994; Ibargüengoytía and Casalins 2007; Table 1). Similarly, other *Phymaturus* species exhibit a variable low litter size of one or two individuals (Habit and Ortiz 1996; Boretto et al. 2007; Boretto and Ibargüengoytía 2009), two to three newborns (Boretto et al. 2014a,b), or a fixed litter size of two individuals (Ibargüengoytía 2004; Boretto and Ibargüengoytía 2006; Cabezas-Cartes et al. 2010). Low mean annual reproductive output has been found in other species in the southern hemisphere (Table 1). According to Méndez-de la Cruz et al. (1993), the maximum litter size (number of follicles recruited for vitellogenesis) is species-specific and determined genetically, whereas litter size in utero is determined by the rate of ovarian atresia, which may be influenced by environmental stimuli. In addition to the atresia of follicles that have already started vitellogenesis, a low number of follicles recruited for vitellogenesis also leads to a reduction in litter size in reptiles (Cree 1994), as we observed in *P. williamsi.*

In contrast to the biennial female reproductive cycle, the spermatogenic cycle in *P. williamsi* can be completed during the warm season, from spring to summer, as was the case of *P. antofagastensis* (Boretto and Ibargüengoytía 2006) and *P. punae* (Boretto et al. 2007). The male reproductive cycle of *P. williamsi* was characterized by the onset of spermatogenesis during spring, when we observed a spermatocyte or spermatids testicular stage, coinciding with an increase in environmental temperatures. Males reach peak gonadal activity, showing abundant sperm in testis and epididymis, in late summer and early autumn, in contrast to *P. zapalensis* (Boretto and Ibargüengoytía 2009), *P. aguanegra* (Cabezas-Cartes et al. 2010), and *P. spectabilis* (Boretto et al. 2014b) that interrupted

and finished the cycle after the brumation period. In *P. williamsi*, we observed males with testicular regression in autumn and after the brumation period in early spring, which would indicate the interruption of spermatogenesis only during this period (mid-autumn to early spring).

According to Pudney (1995), males of *P. williamsi* display a postnuptial reproductive cycle, in contrast to the other species of the *palluma* group that exhibited prenuptial (Boretto et al. 2007), or continuous and asynchronous reproductive cycles (Habit and Ortiz 1996; Boretto and Ibargüengoytía 2006; Cabezas-Cartes et al. 2010). Postnuptial cycles are characterized by spermatogenesis in mid-summer and spermiogenesis from early autumn to the next spring, when mating and ovulation occur, with a temporal dissociation between spermatogenesis and mating, and are common in species that inhabit temperate-cold and unpredictable environments, in which testicular recrudescence occurs after mating, with a consequent storage of sperm in the epididymis (Pudney 1995). This reproductive pattern has been described in other liolaemids, such as *Liolaemus gravenhorsti* (Leyton et al. 1980), *L. elongatus* (Ibargüengoytía and Cussac 1998), and *L. lineomaculatus* (Medina and Ibargüengoytía 2010), and in the *Phymaturus patagonicus* group (Ibargüengoytía 2004; Boretto and Ibargüengoytía 2009; Boretto et al. 2014b). Nevertheless, these *Phymaturus* males do not show sperm reservoir during the brumation period. Our histological analysis indicated that males of *P. williamsi* stored sperm in the male tract during the brumation period through early spring, when mating is more likely to occur, and before testicular recrudescence, increasing the chances of reproduction.

Environmental conditions at high altitudes in the Andes, such as increased intensity of UV radiation, and especially low mean annual temperatures, increased extreme temperatures, and high amplitude of daily temperatures represent an ecological challenge for herpetological communities (Navas 2003) and constrain reproductive cycles (Duvall et al. 1982; James and Shine 1985). These conditions favor the development of reproductive strategies in *P. williamsi* like sperm storage and some degree of asynchrony in the spermatogenic stages at each date of capture, which allow the male reproductive cycle to occur independently from the female cycle. Low environmental temperatures in autumn may induce growth and early testicular spermatogenesis, while warm temperatures would be needed to complete the spermatogenic cycle in spring (Licht et al. 1969). The climatic unpredictability of Andean environments and the characteristics of reproductive cycles of males and females of *P. williamsi* prevented us from finding any relationship between gonadal sizes of both males and

females, and either temperature or rainfall. However, this does not mean that the environmental conditions do not affect the reproductive cycles of *P. williamsi*, given that the beginning of vitellogenesis and spermatogenesis occur when the monthly mean temperature begins to increase in mid-spring, and resume with ovulation and mating the following spring to ensure that embryonic development (gestation) occurs during the months with the best environmental temperature conditions (mid-spring to mid-autumn).

In addition, cold climates in the highlands of the Andes constrains the thermal and energetic requirements for maintenance and reproduction and could drive intersexual differences in the energy invested in reproduction in *P. williamsi*. In this sense, fat body weight of adult males was lower than those of vitellogenic females, and vitellogenic females exhibited higher fat body weight than pregnant females. These results point out intersexual differences in energy use, and that females use the energy obtained by food intake for follicular growth during vitellogenesis, as happens in other liolaemids species of temperate climates such as *L. weigmannii*, *L. huacahuasicus* (Ramírez-Pinilla 1991a,b), and *L. sarmientoi* (Fernández et al. 2015). Indeed, males of *P. punae* invest more energy in growth than females, while females grow less during the vitellogenic process than during pregnancy, supporting the idea that females that allocate one year to each reproductive event (biennial cycle) have differences in the metabolic cost of the reproductive processes (Boretto et al. 2015).

Several traits of the reproductive cycles in the genus *Phymaturus* prove to be highly conservative, like the prolonged female reproductive cycle of *P. williamsi*, the low litter size, and consequently the low mean annual reproductive output. However, the date of births of *P. williamsi* is the latest observed in the genus, and the postnuptial male cycle is more common in the *patagonicus* group of *Phymaturus*. The characteristics of the male reproductive cycle have shown to be more variable within the genus, probably as a result of an adaptation to the female cycle, and to the climatic conditions of each environment. In the highlands of the Andes, the large daily and seasonal thermal fluctuations, the short periods of activity and long periods of brummation associated with food availability and offspring survival influence the requirements of great reproductive efforts in females to increase the survival of newborns, particularly in species with low litter sizes and low mean annual reproductive output like *P. williamsi*. Our work showed that males and females of *P. williamsi* adjust their reproductive activity possibly according to physiological and environmental constraints due to the harsh climate at high altitudes in the Andes. We have also provided information fundamental for population viability analyses with direct relevance to the development of conservation strategies.

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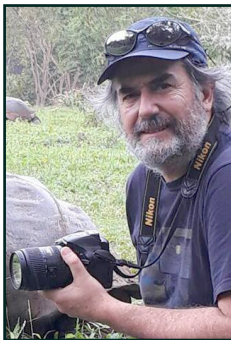
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