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## REPRODUCTIVE BIOLOGY OF *PHYMATURUS SPECTABILIS* (LIOLAEMIDAE): FEMALES SKIP REPRODUCTION IN COLD AND HARSH ENVIRONMENTS OF PATAGONIA, ARGENTINA

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**Abstract.**—Herein, we describe the reproductive biology of *Phymaturus spectabilis*, a lizard species that inhabits rocky outcrops in cold and arid environments of the Patagonian steppe of Argentina. We studied male and female reproductive cycles, mean annual reproductive output, allometric changes during ontogeny, and sexual dimorphism, mainly with a non-invasive methodology used for the first time in Argentina. We corroborated results from this non-invasive method with data from euthanized individuals through observation, classification, and measurement of the female reproductive tract and ovaries and through examination of the histology of testicles and epididymis in males. The presence of males with spermatozoa in the epididymis from early spring and gravid females from mid spring shows that mating, ovulation, and egg development occur at the beginning of the activity season. Parturition occurs in midsummer to two offspring. Our finding that half of the adult females were in a non-reproductive condition during the entire reproductive season, simultaneously with vitellogenic or gravid females, demonstrates that not all of the adult females in this population reproduce every year. Females exhibited an annual to biennial cycle, sometimes skipping a year of reproduction, while males exhibited an annual cycle. *Phymaturus spectabilis* show the common pattern of low mean annual reproductive output found in the genus *Phymaturus*. The low frequency of reproduction and the low mean annual reproductive output are risk factors for this species. These life-history traits, together with its microendemic character (as it is restricted to an area of 70 km<sup>2</sup> of fragmented and rocky plateaus in the poorly protected steppe of Patagonia Argentina), mean that this species is of concern.

**Key Words.**—abdominal palpation; cold and harsh steppe; lizards; reproduction

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### INTRODUCTION

Reproductive biology studies are essential for understanding the life history of a species and have a direct relevance for conservation. This is especially true in the lizards of the *Phymaturus* genus (Liolaemidae). Although the reproductive biology of only six *Phymaturus* species has been studied, their reproductive characteristics and low mean annual reproductive output, together with characteristics of their ecology and distribution, make them vulnerable (Abdala et al. 2012). In addition, while the genus *Phymaturus* is widely distributed in the cold and harsh environments on both sides of the Andean Highlands (Argentina and Chile) and in the arid Patagonian Argentinean steppe (Ceï 1986, 1993), individual species have a very restricted distribution and this genus exhibits significant microendemisms (Abdala et al. 2012). In the last 30 years, the number of known *Phymaturus* species has increased from 10 to 41 (Núñez et al. 2010; Troncoso-Palacios and Lobo 2012; Lobo et al. 2013; Scolaro et al. 2013; Abdala and Quinteros 2014), and there are another 31 populations

under study that may represent new species (Lobo et al. 2012; Morando et al. 2013). However, the reproductive biology of the majority of these species is unknown.

Species of the genus *Phymaturus* show features conserved from the ancestral state, such as their consistent preference for rocky microhabitats, a mainly herbivorous diet, and a viviparous reproductive mode (Ceï 1986, 1993; Scolaro and Iburgüengoytía 2008). The cold and harsh environmental conditions restrict the activity of *Phymaturus* lizards to a limited period from spring to autumn. They spend the rest of the year in dormancy in rock crevices like other Liolaemids lizards from Patagonia Argentina, such as *Liolaemus elongatus* (Iburgüengoytía and Cussac 1998; 1999), *Liolaemus pictus* (Iburgüengoytía and Cussac 1996; 1999), *Liolaemus bibronii*, *Liolaemus boulengeri*, and *Liolaemus lineomaculatus* (Medina and Iburgüengoytía 2010). Most species of *Phymaturus* studied complete a female reproductive cycle in two years (*P. vociferator*: Habit and Ortiz 1996; *P. tenebrosus*: Iburgüengoytía 2004; *P. antofagastensis*: Boretto and Iburgüengoytía 2006; *P.*

*punae*: Boretto et al. 2007; *P. cf. palluma*: Cabezas-Cartes et al. 2010). The only exception occurs in *Phymaturus zapalensis* females, which can reproduce annually but occasionally skip a year of reproduction and therefore have an annual-biennial cycle (Boretto and Ibagüengoytia 2009). The cold harsh environments that *Phymaturus* lizards inhabit, together with the physiological inability of viviparous females to perform vitellogenesis while gravid (Duvall et al. 1982; Callard et al. 1992; Custodia-Lora and Callard 2002), restrict the time available to complete a female reproductive cycle in one year. Males of *Phymaturus* species have developed different ways of adapting to female cycles such as prenuptial, postnuptial, or continuous spermatogenic cycles. In species with prenuptial cycles, males perform spermatogenesis from spring to mid-summer, in synchrony with the follicular development and ovulation of females (*P. vociferator*, Habit and Ortiz 1996; *P. punae*, Boretto et al. 2007). Postnuptial cycles are characterized by the temporal dissociation between spermatogenesis and mating, in which spermatogenesis lasts almost a year from summer, autumn, and winter before resuming the following spring, when mating and ovulation occur (*P. tenebrosus*: Ibagüengoytia 2004; *P. zapalensis*: Boretto and Ibagüengoytia 2009; Boretto et al. 2012). Males with continuous spermatogenic cycles exhibit sperm availability along the entire activity season from spring to autumn (*P. antofagastensis*: Boretto and Ibagüengoytia 2006; Boretto et al. 2010; *P. cf. palluma*: Cabezas-Cartes et al. 2010).

In this study, we examined the reproductive cycles of males and females, minimum adult size, clutch size, allometric changes during ontogeny, and sexual dimorphism of *Phymaturus spectabilis*. This species is a microendemic to an area of approximately 70 km<sup>2</sup> in a volcanic rocky plateau at 1,000 m elevation, close to Ingeniero Jacobacci, in the south-western area of the Río Negro Province, Patagonia Argentina (Lobo and Quinteros 2005; Pincheira-Donoso et al. 2008). In addition, we show the accuracy of the non-invasive technique of abdominal palpation to determine the reproductive stage of adult females in this species with the objective of promoting the use of non-invasive techniques in the study of lizards in Argentina that are vulnerable or at risk of extinction.

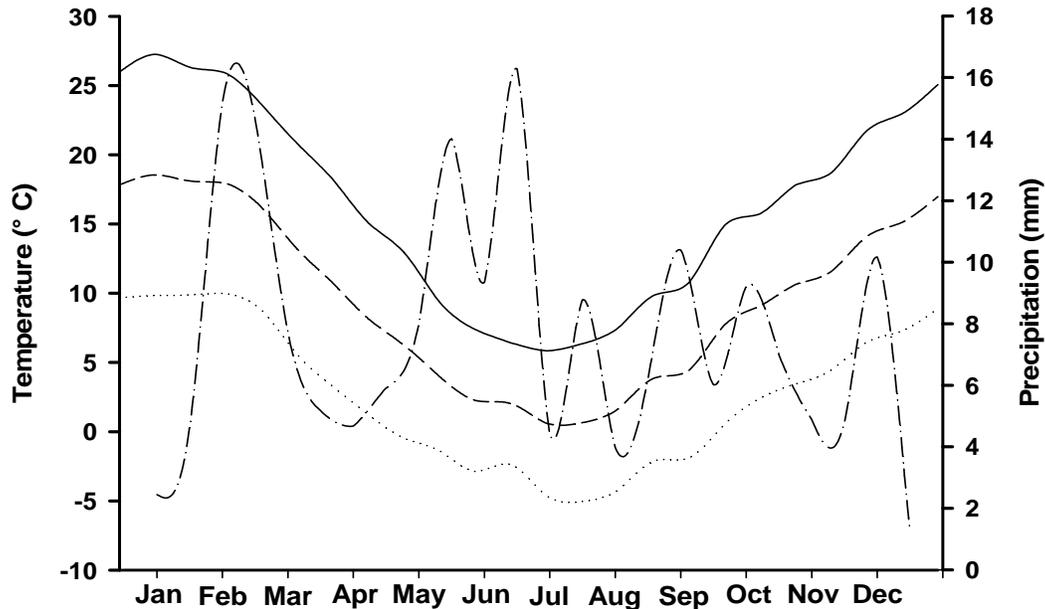
#### METHODS

**Specimens used and capture site.**—We captured *P. spectabilis* lizards from their endemic area, 25 km south of Ingeniero Jacobacci, Río Negro, Argentina (41°S, 69°W at 983–1,064 m elevation). We carried out field work during March 2006, November 2006, October 2007, December 2010, and February–March 2011. During these field trips, we captured by slipknot and georeferenced 178 lizards in granite rock outcrops from 900 to 1900. We weighed (body mass, BM, 100 g spring scale ± 0.5 g; Pesola AG, Baar, Switzerland), determined sex (by the presence of

preloacal pores in males), and measured each specimen (digital gauge ± 0.02 mm, CA-01, Lee Tools, Guangzhou, Guangdong, China) to study the allometric changes during ontogeny and sexual dimorphism. From the total sample of 178 lizards, we classified by abdominal palpation according to the reproductive state (see further explanation below) a subsample of 151 individuals captured during the field trips of December 2010 and February–March 2011. We used GPS data (3 m resolution) to release most lizards at their capture sites immediately following measure and palpation. Two groups of lizards were not immediately released. The first group consisted of 12 gravid females that were transported to the laboratory until parturition (see further explanation below), after which they were released at their original capture sites. The second group consisted of the other 27 lizards of the total sample (17 males and 10 females). We brought these lizards to the laboratory, where they were euthanized by intraperitoneal administration of sodium thiopental anesthetics. They were then fixed in Bouin's solution 24 h and preserved in 70% ethanol to be used to study the spermatogenic stages in males and to corroborate the reproductive state determined by palpation. For this work, we followed the ASIH/HL/SSAR Guidelines for Use of Live Amphibians and Reptiles as well as the regulations detailed in Argentinean National Law #14346. We deposited the specimens in the collection of the Centro Regional Universitario Bariloche of the Universidad Nacional del Comahue.

*Phymaturus spectabilis* habitat is located in the Geomorphologic Region of Endorheic Depressions, in the arid district of the Monte Austral (Godagnone and Bran 2009). The dominant landscape is characterized by barren steppe and dissected rolling plains interspersed with rocky outcrops and vegetation of a shrub-steppe grassy appearance (Cabrera 1971; Godagnone and Bran 2009). There is low herbaceous coverage, composed of sparse cushion bushes (*Stipa papposa*, *S. speciosa*, *S. humilis*, *Poa ligularis*, and *Bromus catharticus*), and subshrubs (*Senecio filaginoides*, *Nassauvia glomerulosa*, and *Grindelia chiloensis*) with bare soil percentages over 50% (Cabrera 1971). We obtained the total daily precipitation (mm), and the maximum, minimum, and mean daily temperature from 1999 to 2011 from the National Meteorological Service of the Argentinean Air Force station, located in Maquinchao 70 km from Ingeniero Jacobacci. We show the biweekly means of the total daily precipitation and the biweekly means of the maximum, minimum, and mean temperature from the last 13 years (Fig. 1).

**Abdominal palpation.**—We palpated each individual (n = 151), which involved gently rolling sections of the lizard's abdomen between thumb and fingers to feel the size and shape of structures within the ventral section of the body following Sinervo and Licht (1991). For each female we registered the



**FIGURE 1.** Climatic conditions from the nearest meteorological station to the study site (Maquinchao, 70 km Northeast). Biweekly means of total mean precipitation (dash-dot) and maximum (solid), minimum (dotted) and mean (long dash) temperatures (°C) from January 1999 to 2011 are presented.

presence/absence of prominent spherical intra-abdominal structures indicative of vitellogenic follicles, or the presence of prominent elliptical structures indicative of intrauterine embryos. Considering the abdominal palpation and the minimum adult size (defined below), we classified the females as juveniles (lack of prominent spherical or elliptical intra-abdominal structures, and snout-vent length [SVL] less than the minimum adult size), adult vitellogenic females (presence of prominent spherical intra-abdominal structures), gravid females (presence of prominent elliptical structures indicative of intrauterine embryos), or non-reproductive adult females (lack of spherical or elliptical intra-abdominal structures, and SVL more than the minimum adult size). For each male, we registered the presence/absence of prominent spherical structures in ventral section of body (indicative of development of testicles) and presence/absence of combat marks. Considering the abdominal palpation and the minimum adult size (defined below) we classified the males as juveniles (lack of prominent spherical structures in ventral section of body, without combat marks, and SVL minor than the minimum adult size) or adults (presence of prominent spherical structures in ventral section of body, and combat marks).

**Female reproductive condition, minimum size at sexual maturity, clutch size, and mean annual reproductive output.**—We dissected the euthanized females ( $n = 10$ ) and determined their reproductive

condition using morphological observations of the ovarian follicles and uteri. Considering that follicles are spherical and that their diameter has been shown to be a reliable variable to study the follicular growth during vitellogenesis, we defined three follicular categories. We used the largest follicular size (Duarte Rocha 1992), measured with a vernier caliper on a camera Lucida scheme ( $\pm 0.1$  mm), and yolk characteristics to classify the follicles as: previtellogenic (small size and absence of yolk), vitellogenic (larger size than previtellogenic follicles, and presence of yolk), or atretic (anomalous distribution of yolk). Finally, based on the integration characteristics of the reproductive tract (uteri) and the follicular categories (*sensu* Ibarquengoytia 2004; Boretto and Ibarquengoytia 2006, 2009; Boretto et al. 2007), we classified the females as juvenile (uteri like a thread, transparent, without folds, and with previtellogenic and small follicles), adult vitellogenic female (distended uteri with medium-size folds over the entire surface, with enlarged vitellogenic follicles), non-reproductive adult (distended uteri with medium-size folds with small vitellogenic follicles), or post-partum adult female (conspicuous and expanded uteri, with numerous and large folds over the entire surface, indicative of recent parturition). We classified the development stage of embryos *sensu* Leyton et al. (1980) from euthanized gravid females ( $n = 2$ ) as: early (from cleavage to somitic embryos), middle (curvate trunk to limbs with five fused fingers, outlines of ears and eyes, and abundant yolk), and late

(with gonadal differentiation, scales and pigmented skin).

We estimated the minimum SVL at sexual maturity based on the size of the smallest female with any of the following traits explained above: follicles with yolk, embryos in uteri, or distended and folded uteri. To estimate the dates of parturition and the clutch size in *P. spectabilis*, during January-February 2011, we transported 12 gravid females to the laboratory and maintained them in the laboratory until parturition. We provided females with food and water *ad libitum*, in an open-top, glass terrarium (117 × 40 × 50 cm) with sand and rocks from the site of capture, UV lamp (Sylvania-Reptistar®, Erlangen, Bavaria, Germany), and infrared lamp (150W, General Electric®, Budapest, Central Hungary, Hungary) on one side to provide a temperature gradient. Air temperature in the room ranged from 18.8–23.1° C. We estimated the clutch size by counting the total number of embryos present in the right and left uterus of each female euthanized, and the number of offspring born in captivity from each female. We calculated the mean annual reproductive output as the product of clutch size and clutch frequency (1: annual female reproductive cycle; 0.5: biennial reproduction; *sensu* Cree 1994; 0.75: annual-biannual reproduction; *sensu* Boretto and Ibarngüengoytia 2009).

**Male reproductive condition and sexual maturity.**—We measured the antero-posterior diameter of testis of euthanized males (n = 17) with a vernier caliper on a camera Lucida scheme (± 0.1 mm). To study the reproductive cycle of males, we removed the right testis and epididymis and dehydrated in an ethanol series, and embedded in paraffin. We stained the sections (7 µm) with Hematoxylin and Eosin (Martoja and Martoja Pierson 1970). We defined the stages of spermatogenesis *sensu* Mayhew and Wright (1970) as: (1) only spermatogonia; (2) primary and/or secondary spermatocytes; (3) spermatids; (4) spermatozoa in tubular lumen and in the epididymis; or (5) regression, with scarce spermatozoa in tubular lumen and sperm in epididymis. We determined the minimum SVL at sexual maturity in males based on the size of the smallest individual showing spermatogenesis or spermatozoa in epididymis.

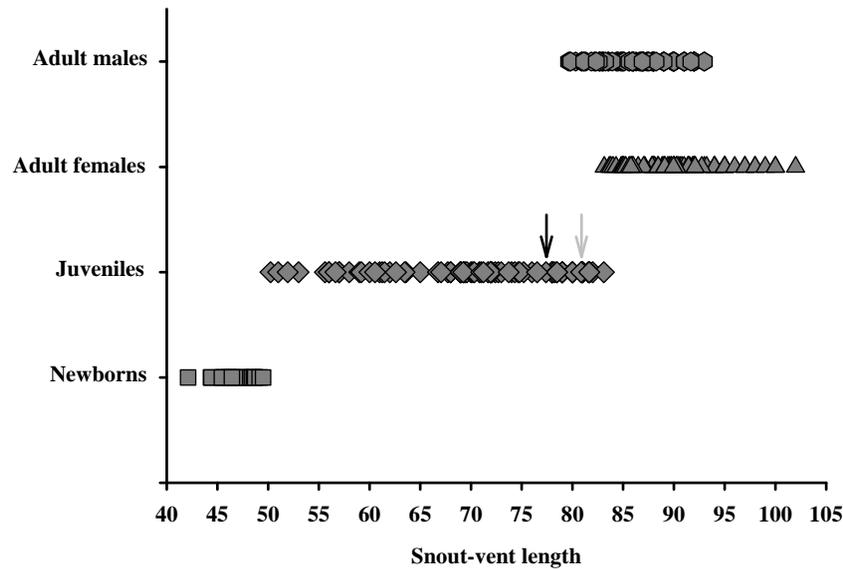
**Allometric changes during ontogeny and sexual dimorphism.**—For analyses of allometry, we included newborns (born in the laboratory, n = 20) and juveniles (n = 53). We restricted the morphological descriptions for the assessment of sexual dimorphism only to adult males (n = 57) and females (n = 63) based on the minimum adult sizes estimates from euthanized individuals. We used a digital gauge (± 0.2 mm, Lee Tools, Guangzhou, Guangdong, China) to measure the following variables: SVL, head length (HL), head width (HW), neck width (NW), width of the tail base at the vent (WTV), maximum body width

(BW, gravid females not included), diameter of the front leg (FLD) and diameter of the hind leg (HLD) at the insertion with the shoulders and pelvic girdles, respectively, right and left distance between front and hind limbs (inter-limb length, IL), and hip width (HipW) measured as the body width at the insertion of hind legs.

**Statistical analyses.**—We used the statistical software Sigma Stat 3.5® (Systat Software Inc., Chicago, Illinois, USA), SPSS 17.0® (Chicago, Illinois, USA), and Sigma Plot 10.0® (Systat Software Inc., Chicago, Illinois, USA). All data met the assumptions for parametric analysis unless otherwise stated. For the analysis of the differences in SVL between adult males and females, we used a t-test. When the data did not meet the assumptions of normality and homogeneity of variance required for parametric tests, we used the Mann-Whitney test. Furthermore, to avoid size dependence in the analysis of sexual dimorphism, we used the residuals from a regression of the natural logarithms (ln) of each morphological variable against ln(SVL) in a Discriminant Analysis. We analyzed the allometric changes along ontogeny by doing the regression of the ln of each morphometric variable and ln(SVL) in juveniles, adult males and adult females. For the analysis of the relationship between SVL and follicular or testicular size, or between reproductive qualitative features in males, we used Linear Regressions or Pearson and Spearman correlations. We used the Paired t-test to analyze the differences between right and left testicular size, and the binomial test to analyze the differences in the proportion of reproductive versus non-reproductive females, and in the proportion of reproductive-receptive males and females. We tested the assumptions of normality and homogeneity of variance with the one-sample Kolmogorov-Smirnov test and with the Levene test, respectively (Sokal and Rohlf 1969). Means are given ± SE and for tests, α = 0.05.

## RESULTS

**Minimum adult sizes, sexual dimorphism and allometric changes along ontogeny.**—The abdominal palpation allowed us to determine the minimum adult size of female *P. spectabilis* as 83.6 mm SVL, which presented prominent elliptical structures indicative of intrauterine embryos. The smallest euthanized female that showed signs of an adult reproductive condition, namely a distended uterus with medium-size folds over the entire surface, had a SVL of 83.1 mm. The minimum adult size of a male showing reproductive activity recognized by palpation was 77.4 mm SVL and in a euthanized male that showed spermatozoa testicular stage was 79.6 mm SVL. In consequence, we considered for the reproductive cycles, and for the allometric and dimorphic studies that the minimum



**FIGURE 2.** Snout-vent length of newborns (squares), juveniles (diamonds), adult females (triangles), and adult males (circles) of *Phymaturus spectabilis*. The arrows indicate the minimum adult sizes obtained by abdominal palpation classifications for males (black arrow) and females (gray arrow).

adult SVL in females was 83.1 mm, and in males was 79.6 mm (Fig. 2).

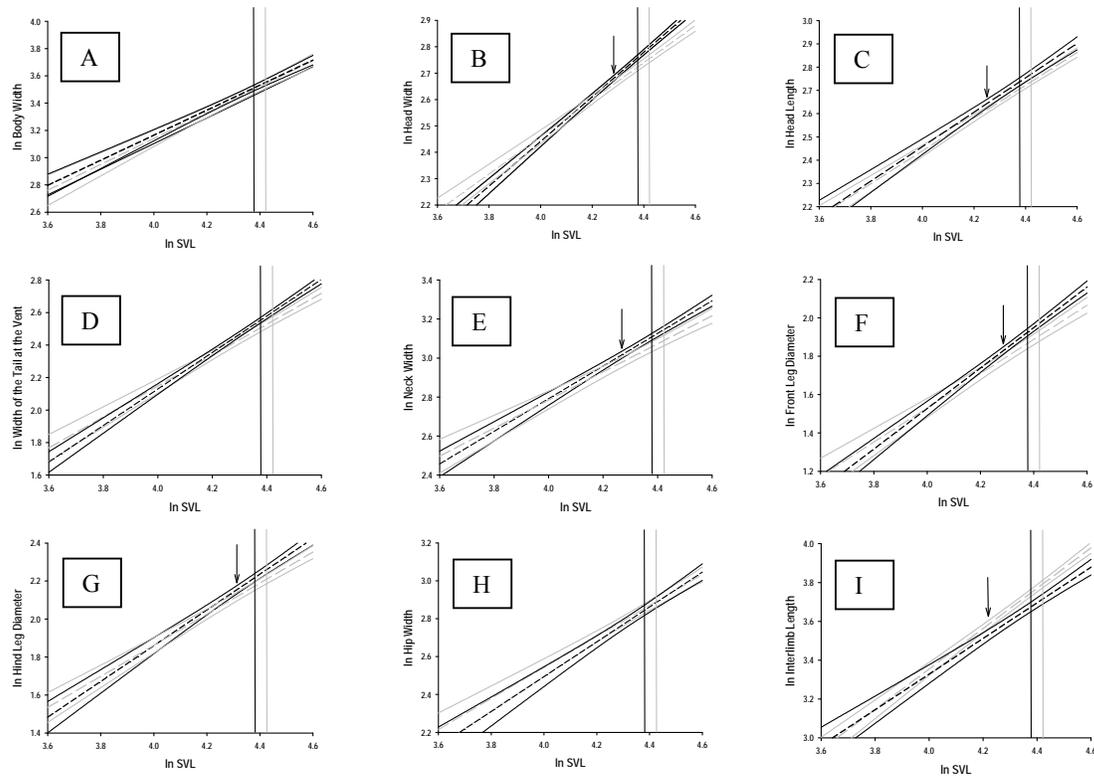
Comparison between males and females showed that females exhibited significantly larger SVL ( $n_{\text{females}} = 87$ ,  $n_{\text{males}} = 85$ ;  $U = 5,463.0$ ,  $P < 0.001$ ;  $\text{mean}_{\text{females}} = 89.8 \pm 0.5$  mm;  $\text{mean}_{\text{males}} = 86.0 \pm 0.4$  mm) and inter-limb length than males, whereas males exhibited larger head width than females (Discriminant Analysis,  $\lambda = 0.759$ ,  $\chi^2 = 12.679$ ,  $df = 2$ ,  $P < 0.002$ ). Confidence intervals of the allometric regressions of morphometric variables with SVL showed that HW, HL, NW, WTV, FLD, and HLD have a significantly higher growth rate in adult males than in females after sexual maturity, while females showed a significantly higher growth rate only in IL ( $P < 0.050$ ; Fig. 3).

**Female reproductive cycle, clutch size, and mean annual reproductive output.**—In early spring, we captured one adult female at the early vitellogenic stage with its largest follicle measuring 3.6 mm, and two post-partum females, with their largest follicle size of 4.6 mm and 2.9 mm respectively (Fig. 4A). In mid spring, we captured one non-reproductive female with its largest follicle size of 2.2 mm, and two gravid females with embryos in early stages of development (Fig. 4A). From late spring to midsummer, we captured 49 juvenile females and 48 adult females classified by ventral abdominal palpation, and 58% of the adult females ( $n = 28$ ) were reproductive (advanced vitellogenesis or gravid), whereas the other 42% ( $n = 20$ ) were non-reproductive (Fig. 4A). The gravid females captured with external signs of advanced gravidity ( $n = 12$ ), and maintained in

captivity until parturition in midsummer (third and fourth week of February) to two offspring each. In addition, in the field we captured newborn lizards also in midsummer (47.5–49.5 mm SVL), and juveniles with small SVL were captured in spring (range<sub>SVL</sub> = 50.2–53.0 mm,  $n = 3$ ), confirming that in the field, parturition occurs during the same period of time (midsummer) that were observed in the laboratory. Females of *P. spectabilis* exhibited a fixed clutch size of two offspring per female.

In early autumn, we captured two non-reproductive females that exhibited small vitellogenic follicles measuring 3.0 and 3.6 mm respectively, and two vitellogenic females with their largest vitellogenic follicles measuring 6.0 and 11.9 mm, respectively (Fig. 4), and distended uteri with medium-size folds indicative of the medium vitellogenic stage. Considering both euthanized and palpation methodologies, *Phymaturus spectabilis* did not exhibit significant differences in the general proportion of reproductive (follicles larger than 4 mm or gravid;  $n = 36$ , 59%) and non-reproductive females (follicles smaller than 4 mm or non-reproductive by abdominal palpation;  $n = 25$ , 41%; Binomial Test,  $P = 0.200$ ,  $n = 61$ ). The SVL of reproductive and non-reproductive females did not differ significantly ( $\text{mean}_{\text{SVL reproductive}} = 91.1$  mm;  $\text{mean}_{\text{SVL non-reproductive}} = 90.3$  mm;  $t = -0.684$ ,  $df = 59$   $P = 0.496$ ,  $n = 61$ ).

**Male reproductive cycle.**—Right versus left testicular size of adult males did not differ significantly ( $t = 1.255$ ,  $df = 15$ ,  $P = 0.229$ ,  $\text{mean}_{\text{right}} = 7.6 \pm 0.6$  mm,  $\text{mean}_{\text{left}} = 7.4 \pm 0.5$  mm,  $n = 16$ ).



**FIGURE 3.** Allometric relationships of the natural logarithms (ln) of all morphometric variables with SVL, for male and female *Phymaturus spectabilis*. Regression and confidence intervals are indicated (black: males; gray: females). Vertical lines indicate SVL at sexual maturity determined by gonadal index and histology of gonads (black: males; gray: females), the arrows indicate when a morphometric variable differs between males and females. A: ln maximum body width versus ln SVL; B: ln head width versus ln SVL; C: ln head length versus ln SVL; D: ln width of the tail base at the vent versus ln SVL; E: ln neck width versus ln SVL; F: ln front leg diameter versus ln SVL; G: ln hind leg diameter versus ln SVL; H: ln hip width versus ln SVL; I: ln interlimb length versus ln SVL.

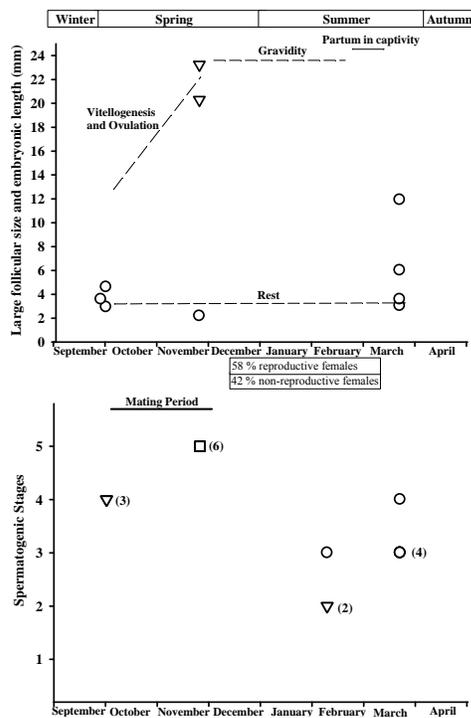
Consequently, we arbitrarily chose the right testes for the histological analyses. In the *P. spectabilis* adult males, there were no significant relationships between testicular size and SVL ( $\text{Log}_{10}$  transformed;  $F_{1,16} = 1.243$ ,  $P = 0.282$ ), but there was a significant relationship between testicular size and spermatogenic stage ( $r = 0.882$ ,  $P < 0.001$ ,  $n = 17$ ).

At the beginning of spring males exhibited spermatozoa in testis and epididymis (stage 4,  $n = 3$ ), and in mid spring they had testis in regression and abundant spermatozoa in the epididymis (stage 5,  $n = 6$ ; Fig. 4B). In midsummer males began a new spermatogenic cycle, showing spermatocytes (stage 2,  $n = 2$ ) or spermatids in testis (stage 3,  $n = 1$ ), some of the individuals had traces of sperm in the epididymis, from the previous cycle. In early autumn some males exhibited spermatids (stage 3,  $n = 4$ ) and another exhibited spermatozoa in tubular lumen (stage 4,  $n = 1$ ), none of them had sperm in epididymis (Fig. 4B).

**Operative sex ratio.**—There were significant differences in the proportion of reproductive males-receptive females (Proportion reproductive males – receptive females = 65–35; Binomial Test,  $n = 95$ ,  $P < 0.004$ ).

## DISCUSSION

Females of *P. spectabilis* can display an annual cycle in which vitellogenesis begins in autumn and finishes in mid spring and gravidity occurs from late spring to midsummer. Nevertheless, the fact that the proportion of non-reproductive females did not significantly deviate from 50% during the activity season indicates that some females skip of a year of reproduction, and consequently, have a biennial reproductive cycle. The presence of hypertrophied uteri with medium-size folds over the entire surface characteristic of estradiol activity and sexually mature females (Van Wyk 1991) allowed us to confirm that these individuals were adult females of *P. spectabilis*. In addition, we can also discard the possibility that there is a displacement of smaller females by larger individuals as the non-reproductive adult females are not significantly smaller than those adult females, which are in the vitellogenesis or gestation stage. Based on these different lines of evidence, we defined the female reproductive cycle of *P. spectabilis* as annual-biennial. Skipping a year has also been described for other species from diverse taxa lineages living in harsh environments, such as the lizard *Cordylus giganteus* (South Africa; Van Wyk 1991), the snakes *Arizona elegans* and *Crotalus viridis* (New



**FIGURE 4.** Reproductive cycle of males and females of *Phymaturus spectabilis*. A: Large follicular sizes (mm; circles) of dissected adult females and length of embryos (mm; triangles) from dissected gravid females versus date. The inferior bars indicate the period of time when females were captured and palpated and the percentage of reproductive and non-reproductive females determined by abdominal palpation. The dotted lines indicate the period of time when females undergoing each phase in the reproductive cycle according to the information of dissected females (triangles and circles data points) and palpated females (bars). B: Spermatogenic stages versus date of juveniles (spermatogenic stage 1) and adult males (spermatogenic stages 2 to 5) with abundant (square), moderate (triangle), or without spermatozoa in epididymis (circle). Brackets indicate the number of observations.

Mexico; Aldridge 1979), the water skink *Eulamprus tympanum* (Schwarzkopf 1993) and the scincid *Niveoscincus microlepidotus* (Australia; Olsson and Shine 1998, 1999), among others. Skipping reproduction has been also observed in Patagonia in the lizards *Liolaemus pictus* (Ibargüengoytia and Cussac 1996), and *P. zapalensis* (Boretto and Ibargüengoytia 2009), and in the nocturnal gecko *Homonota darwini* (Ibargüengoytia and Casalins 2007).

Males of *P. spectabilis*, however, exhibited an annual reproductive cycle synchronous with that of reproductive females. Males initiate the spermatogenic cycle in midsummer, and spermatogenic stages 3 (spermatids) and 4 (spermatozoa) are present from early autumn to the next spring. The presence of males with spermatozoa in epididymis at the beginning of spring and the presence of females at early gravidity at the end of spring strongly support

our hypothesis that mating and ovulation in *P. spectabilis* occur during the first half of this season. The same reproductive cycles have been observed in other *Phymaturus* that inhabit the Patagonian steppe of Argentina, as *P. tenebrosus* (Ibargüengoytia 2004) and *P. zapalensis* (Boretto and Ibargüengoytia 2009) pointing out that there is a reproductive pattern of postnuptial male cycles and annual to biennial female cycles among the *P. patagonicus* group, which differs from the *P. palluma* group (Boretto and Ibargüengoytia 2009). Females from the *P. palluma* group are strictly biennial and males have exhibited different reproductive cycles from prenuptial to continuous and asynchronous (Habit and Ortiz 1996; Boretto and Ibargüengoytia 2006; Boretto et al. 2007; Cabezas-Cartes et al. 2010).

In addition, *P. spectabilis* showed sexual dimorphism characterized by a larger SVL in females than males and a larger head width in males than females, as in the other species studied from the *P. patagonicus* group (Ibargüengoytia 2004; Boretto and Ibargüengoytia 2009), whereas a larger SVL in males than females has been observed in all species of the *P. palluma* group (Boretto and Ibargüengoytia 2009; Cabezas-Cartes et al. 2010). The larger heads in males of *P. spectabilis* may have arisen through sexual selection, in order to enhance success in male-male fights (Ibargüengoytia and Cussac 1999; Boretto and Ibargüengoytia 2006), as a consequence of the male biased operational sex ratio, due to the low proportion of reproductive females in the population. The larger inter-limb length observed in *P. spectabilis* females has been previously observed in all *Phymaturus* studied, supporting that this dimorphic trait (larger in females than in males) is a characteristic of the genus (Boretto and Ibargüengoytia 2009; Cabezas-Cartes et al. 2010; present study). In several species, a larger inter-limb length in females can be attributed to fecundity selection for increased space to hold the developing eggs or embryos (Andersson 1994, Olsson et al. 2002), increasing not only the number of offspring, but also offspring size (e.g., Shine 1992; Qualls and Shine 1995). Future studies will confirm whether in *Phymaturus* there is a strong selection regarding embryo size or energetic reserves in newborns related to a larger inter-limb length, SVL or body width, a common pattern when the brood size is small (Olsson et al. 2002). In addition to the small clutch size (fixed at two newborns) and the prolonged annual-biennial female reproductive cycles, *P. spectabilis* exhibited a low mean annual reproductive output of 1.5 ( $2 \times 0.75$ ), and output for the other *Phymaturus* (0.75: *P. vociferator*, *P. punae*, *P. cf. palluma*; Habit and Ortiz 1996; Boretto et al. 2007; Cabezas-Cartes et al. 2010; 1:0 *P. tenebrosus*, *P. antofagastensis*; Ibargüengoytia 2004; Boretto and Ibargüengoytia 2006; 1.12: *P. zapalensis*; Boretto and Ibargüengoytia 2009) are among the lowest for reptiles in the world (Cree 1994; Ibargüengoytia and Casalins 2007).

In spite of the differences in the timing of female reproductive cycles among *Phymaturus* phylogenetic groups, in all the species studied, parturitions occur during mid to late summer, revealing once again, the constraints that harsh environmental conditions have on the timing of reproduction. The cold environments of the Andes highlands and the Patagonian Argentina where *Phymaturus* inhabit, together with hormonal and physiological restrictions inherent to viviparity (Callard et al. 1992; Custodia-Lora and Callard 2002), prevent females from completing vitellogenesis, gravity, and storing fat within one activity season (Boretto et al. 2007; Boretto and Ibargüengoytía 2009; Cabezas-Cartes et al. 2010). Under this scenario, when the length and thermal quality of the activity season are variable, flexibility in frequency of reproduction seems to be an efficient mechanism to cope with unpredictable climates (Boretto and Ibargüengoytía 2006, 2009). When the activity seasons are relatively cool and/or short and postpartum feeding opportunities are limited, females devote the following season to growing and storing reserves (e.g., capital breeding) for future reproductive bouts, but under benign conditions reproduction can be resumed in a year (Saint Girons 1985; Schwarzkopf 1993; Cree 1994; Ibargüengoytía and Cussac 1996; Boretto and Ibargüengoytía 2009). Parental “allocation decisions” that increase an offspring’s probability of survival through the earliest stages of their life cycle are under strong selection pressure (Sinervo et al. 1992), and may counteract the low frequency of reproduction resulting from multiannual reproductive cycles. The abundant fat reserves observed in the offspring of *P. spectabilis*, as well as the high parental investment per offspring denoted by the large fat bodies and intra-abdominal yolk reserves found in the offspring of *P. punae* (Boretto et al. 2007), *P. antofagastensis*, *P. cf. palluma* (Cabezas-Cartes et al. 2010) and *P. zapalensis* (Boretto and Ibargüengoytía 2009), reinforces this prediction, because these reserves could enhance offspring survival, even when the offspring’s investment might be detrimental for future reproduction of females (Boretto et al. 2007; Boretto and Ibargüengoytía 2009; Cabezas-Cartes et al. 2010).

The *P. patagonicus* group is especially vulnerable because the isolated populations are restricted to rock promontories of only severely fragmented habitats in a limited geographic range (approximately 700 km<sup>2</sup>; Piantoni et al. 2006; Abdala et al. 2012). In addition, the habitat is going through a desertification process, a significant anthropogenic impact caused by the over-exploitation of the land, especially by sheep ranching and shrub fires often occur in summer (Piantoni et al. 2006), aggravated by global climate change (Sinervo et al. 2010). As a consequence, *Phymaturus* populations are showing retraction and some may have become extinct, probably because of their viviparous condition and their preference for low body temperatures (Ibargüengoytía and Casalins 2007). For

example, the type locality of *Phymaturus manuelae* population is already locally extinct (pers. obs.).

Herein, we established the efficacy of non-invasive methodologies for the study of reproductive biology in *P. spectabilis*, particularly important due to the vulnerable conservation status of the species and the genus (Abdala et al. 2012). The knowledge of the patterns of reproduction, and specially the rates of reproduction, are relevant for conservation. The possibility of studying the reproductive biology of species using non-invasive techniques is a critical tool. Abdominal palpation used in *P. spectabilis* is an effective technique to discriminate juveniles from adults, reproductive stages of adult females and size at sexual maturity, and confirmed the plasticity of an annual-biennial female cycle without the need to euthanize a larger number of lizards. Indeed, the minimum adult size determined by palpation, was supported by the allometric changes observed between juvenile and adults. In summary, it is necessary to develop non-invasive programs and bio-ecological studies of this poorly known genus to protect the herpetofauna of Patagonia.

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