An Acad Bras Cienc (2022) 94(Suppl. 4): e20210179 DOI 10.1590/0001-3765202220210179
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ANIMAL SCIENCE

High reproductive effort in a vulnerable lizard from high altitudes in Argentina: Reproductive biology and sexual dimorphism in *Phymaturus extrilidus*

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Abstract: Reproductive biology is fundamental to understanding the ecology and evolution of lizards which, in turn, is essential for the definition of the species´ conservation status. We studied life-history traits related to the reproduction of the *Phymaturus extrilidus* lizard, including the male and female reproductive cycles, litter size, mean annual reproductive output, reproductive effort, sexual maturity size and sexual dimorphism, body condition, and fat body cycles. We found sexual dimorphism in size and shape, supporting the hypotheses of sexual and fecundity selection. Females exhibited biennial reproductive cycles synchronous with the annual prenuptial male cycle, adjusted for the maturation of the vitellogenic follicles of females. Females of *P. extrilidu*s have the highest mean annual reproductive output (MARO=1.14) recorded in *Phymaturus*, and this is accompanied by the highest reproductive effort (C=0.28, $C_{energetic}$ =0.31). Births occur from late summer to early autumn. The female reproductive cycle, strictly biennial, like all species of the *P. palluma* group, and the vitellogenesis in particular, appear to be limited by body condition and the amount of fat body stored. This study presents the fundamental reproductive traits of *P. extrilidu*s that can provide valuable information to be used in the evaluation of the conservation status of this species.

Key words: Biennial reproduction, body condition, life-history, viviparity.

INTRODUCTION

Reptiles display great diversity in their life-history traits and particularly in their reproductive output (Shine 2005). Reproduction and life-history are fundamental for the study of lizard ecology and evolution and, ultimately, are essential for conservation (Cree & Hare 2016). Life-history patterns have been described along a continuum between "slow" life histories in mainly viviparous species with low reproductive rate, slow development, late sexual maturity and long life span (longevity), which generally inhabit cold environments, and "fast" life histories occurring mainly in oviparous species with small body size, early sexual maturity, multiple broods per season, and that in general inhabit tropical environments (Tinkle et al. 1970, Dunham & Miles 1985, Ricklefs & Wikelski 2002, Shine 2005, Mesquita et al. 2016). The adaptation to certain environments often entails trade-offs between life-history traits that can make species vulnerable when facing new environmental changes, such as the reproductive mode (oviparous or viviparous; Jara et al. 2019, Ibargüengoytía et al. 2021), more specialized habits (e.g. diet and habitat; Angermeier 1995, McKinney 1997, Foufopoulos

& Ives 1999); the slowdown of life histories (Ducatez & Shine 2019, Turvey & Crees 2019); and low vagility (Mac Nally & Brown 2001). At a population level, the abundance limiting traits could include a restricted distribution (Gaston 1994), small population size (Pimm et al. 1988, Caughley 1994, Belovsky et al. 1999), and low genetic variation (McKinney 1997).

Several of these individual and population traits are present in the *Phymaturus* genus that is composed of a group of specialized and viviparous lizard species that inhabit rocky promontories in cold environments at high altitudes in the Andes mountains in northern Argentina and Chile and volcanic plateaus in Patagonia, southern Argentina (Morando et al. 2013, Lobo et al. 2016, 2018) from 25° to 45° south (Hibbard et al. 2018). Despite the wide distribution range of *Phymaturus*, most species inhabit very restricted habitats with a high degree of endemism among lineages (Andrade-Díaz et al. 2017), and a common pattern of slow life histories, characterized by slow growth rates, high levels of bone resorption, delayed sexual maturity, and high longevity (Piantoni et al. 2006, Boretto et al. 2015, 2018, Cabezas-Cartes et al. 2015, 2018). The *Phymaturus* genus is divided into two main clades, the *P. palluma* and the *P. patagonicus* groups (Etheridge 1995) in which studies of reproductive biology and life-history traits reveal two distinct patterns of physiological trade-off between growth and reproduction (Boretto et al. 2015, 2018). The first of these, exhibited by species within the *P. palluma* group, consists of females that invest more time in reproduction but expend less energy and biomass in each reproductive event (Boretto et al. 2015, 2018). In contrast, the other pattern exhibited by species within the *P. patagonicus* group is notable for females that invest a smaller amount of time in reproduction that is compensated by high reproductive frequencies and high investment of energy and biomass in each reproductive event (Boretto et al. 2018). These patterns indicate the existence of a physiological trade-off between the number of reproductive events throughout the life cycle and the reproductive effort devoted to each one (Boretto et al. 2018).

In addition, *Phymaturus* exhibit low litter size and one of the lowest mean annual reproductive outputs (MARO, *sensu* Cree 1994) relative to other lizards (Ibargüengoytía & Casalins 2007, Castro et al. 2018), as a consequence of the prolonged female reproductive cycle (annual-biennial or strictly biennial; Habit & Ortiz 1996, Ibargüengoytía 2004, Boretto & Ibargüengoytía 2006, 2009, Boretto et al. 2007, 2014a, Cabezas-Cartes et al. 2010, Castro et al. 2018). The slow life-history of *Phymaturus* species, along with scarce populations, and high degrees of endemism, result in the categorization of the whole genus in a Vulnerable conservation status (Abdala et al. 2012), and includes some species that have been recently categorized by the IUCN as Endangered (*Phymaturus tenebrosus*, Abdala 2017).

The low frequency of female' reproduction described in *Phymaturus*, that occurs because of their prolonged cycles and the thermal restrictions of the cold environments they inhabit, result in complex causal relationships between female and male reproductive cycles that also affect male dimorphic traits (e.g. Ibargüengoytía 2004, Boretto & Ibargüengoytía 2009, reviewed in Boretto et al. 2020). *Phymaturus* males, therefore, have evolved several types of reproductive cycles to coordinate with the short female reproductive season and the low frequency of receptive females (Boretto et al. 2020). Three types of cycles have been observed in *Phymaturus* lizards; prenuptial cycles in which spermatogenesis occurs from spring to mid or late summer in synchrony with follicular development; postnuptial cycles where spermatogenesis occurs from mid-summer to autumn and the sperm is then stored until the following spring when mating occurs; and continuous cycles where males have sperm in the seminiferous tubules and/or in the epididymis throughout the entire active season (reviewed in Méndez de la Cruz et al. 2014, Boretto et al. 2020). The three types of cycles have been described in the *P. palluma* group, while in the *P. patagonicus* group, only postnuptial spermatogenic cycles have been observed (Boretto et al. 2020).

Sexual size dimorphism has been analyzed from the perspective of three important types of pressures: fecundity selection, sexual selection, and natural selection (Cox et al. 2007). Body size and shape are essentials to understanding the phenotypes associated with selection pressures in lizards. In females, larger body size and the shape of specific traits than males, taken together with a high energy allocation to reproduction could be attributed to a strong fecundity selection to increase litter or offspring size (Vitt & Congdon 1978, Olsson et al. 2002, Werneck et al. 2009). However, the relation between body size and shape male-biased, could be attributed to strong sexual selection under male-male rivalry and reproductive success, as has been reported for several lizard species (Tokarz 1995, Olsson et al. 2002, Cox et al. 2003). In *Phymaturus*, studies of sexual dimorphism from a body size perspective (SSD) reveal two distinctive patterns; males with larger snout-vent length (SVL) and head size than females in the species of the *P. palluma* group, suggesting strong sexual selection (Boretto & Ibargüengoytía 2009), and females with larger SVL than males and interspecific variation in head size of males, suggestive of selection toward a higher reproductive output in the *P. patagonicus* group (Boretto & Ibargüengoytía 2009, Valdecantos et al. 2019). However, sexual dimorphism in body shape traits (SShD) and its relationship with reproductive biology was not yet been addressed for this genus. The analyses of sexual pressures on shape dimorphism provide an alternative to understanding the selection on morphological traits in the absence of selection for differences in body size (Schwarzkopf 2005).

In recent decades, the *Phymaturus* genus has been expanded considerably (Abdala & Quinteros 2014) and is currently composed of 50 recognized species (Lobo et al. 2021). The reproductive biology, however, has only been studied for 16% of the species of the genus. The understanding of key functional traits including body size, life-history, and demography is fundamental to determining the risks of species extinction (Miles 2020). The limited information available on fundamental functional traits impedes precise definition of the threatened status or extinction risk of species (Miles 2020) resulting in Data Deficient and Unassessed species classification by the IUCN for the majority of *Phymaturus* species.

Herein we study the reproductive biology of *Phymaturus extrilidus*. This species is a microendemic lizard from Sierra de la Invernada, Ullum, San Juan, Argentina (Lobo et al. 2012) that belongs to the *P. palluma* group. We address the following questions: (1) Does the female reproductive cycle follow the conservative biennial pattern of reproduction described for the *P. palluma* group? (2) Are female and male reproductive cycles synchronous? (3) When do births occur? (4) Are males larger than females and different in body shape? (5) Are there differences in the body condition index and fat body cycle between females and males associated with reproduction? We provide valuable information on the reproductive biology that contributes to the knowledge of the reproductive strategies of lizard living in harsh environments and to the precise evaluation of the conservation status of this particular species.

MATERIALS AND METHODS

Specimens used and study area

We studied preserved specimens of *P. extrilidus* from the herpetological collection of the Universidad Nacional de San Juan (FCEFyN - UNSJ, see Appendix I) collected during April 2014 (early autumn; N_{total} = 42, $N_{females}$ = 19, N_{males} = 19, and $N_{newborns}$ = 4) and December 2014 (late spring; N_{total} = 40, $N_{females}$ = 18, and N_{males} = 22) in the Reserve Don Carmelo. In the same site where the original collection specimens were obtained, we collected by hand or noose a subsample in October 2016 (mid-spring; N_{total} = 14; $N_{females}$ = 7, N_{males} = 7), in order of obtain more accurate information about the reproductive stages of males and females during the spring, when lizards from cold climates initiate post-brumation activity. The Don Carmelo Reserve, in the Sierra de la Invernada, Ullum department, San Juan province, Argentina (30° 56′ 59″ S, 69° 04′ 50″ W; 3100 m asl) is a protected area that belongs to the Puna phytogeographic province where the climate is hyper-arid desert (Köppen 1931) and strongly seasonal, with low mean annual temperatures (9.33 °C; Figure 1). Rainfall occurs mainly during the summer season, with prolonged snowfalls from mid-autumn to early spring (Data obtained from Climatic Database 3CN, CIMA/CONICET-UBA, Figure 1). The lizard capture and euthanization methods followed the ASIH/HL/SSAR Guidelines for Use of Live Amphibians and Reptiles as well as the regulations detailed in the Argentinian National Law #14346. Ethical approval Acta N° 30, Exp. 02-2241-P-16 from the Universidad Nacional de San Juan.

After the capture lizards were weighed (body mass, BM, 100 g spring scale ± 0.5 g; Pesola ®), euthanized by an overdose of xylocaine 2% (Dultriec ©), fixed in Bouin's solution for 24 h, and preserved in ethanol (70%) at the herpetological collection of the Facultad de Ciencias Exactas Físicas y Naturales of the Universidad Nacional de San Juan (Appendix I).

A total sample of 96 specimens (35 adult females, 44 adult males, 13 juveniles, and 4 newborns) were measured (snout-vent length, SVL, 150 mm ± 0.2 Diamante ® stainless ruler). We excised and weighed the fat body of each lizard with an analytical electronic balance (± 0.0001 g, AGZN200, Torbal, Poland). The SVL and BM were then used to calculate the scaled mass index ($\hat{M}_i$, *sensu* Peig & Green 2009, 2010) to analyze the body condition of each individual. According to Peig & Green (2009) the body mass index $\hat{M}_i$ is the energy capital accumulated in the body as a result of feeding, and is assumed to be an indicator of an animal's health and quality. We calculated the $\hat{M}_i$ of each adult male, non-pregnant adult female, and juvenile individual and interpreted it as an estimate of the energy store or fat reserve related to fitness (Peig & Green 2009, 2010). The ($\hat{M}_i$) was calculated as $\hat{M}_i = BM_i \ X \ [SVL_0/SVL_i]^{b^{SMA}}$ where BM_i and SVL_i are the body weight and snout-vent length of the individuals, SVL_0 is the arithmetic mean SVL of the population, and b^{SMA} is the standardized major axis slope from the regression of ln(BM) on ln(SVL) for the population (Peig & Green 2009, 2010). To analyze the seasonal variation in the energy store of males and females we broke the seasons down into periods according to sample date (October sample = mid-spring; December sample = late spring; April sample = early autumn). We also analyse the variation in the energy store of males and females considering the reproductive stages.

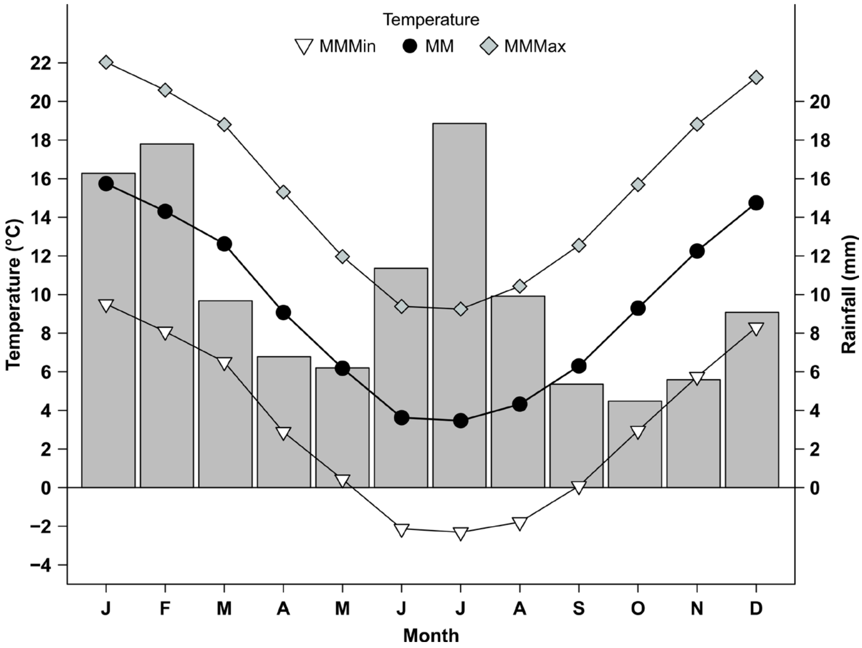

Figure 1. **Mean monthly rainfall (mm, gray bars) and temperatures (°C, polygons: mean monthly temperatures black circles [MM], mean monthly minimum temperatures, white triangles [MMMin], and mean monthly maximum temperatures, grey diamonds [MMMax]) at Sierra de la Invernada (1960 - 2010). Intersections between polygons and bars indicate moisture surplus during winter. Climatic data provided by 3CN database (Centro de Investigaciones del Mar y Atmósfera).**

Morphometry and sexual dimorphism

We assessed sexual dimorphism only in adult individuals ($N_{total} = 63$, $N_{females} = 27$, $N_{males} = 36$) using the following morphometric traits: SVL, HL (head length) from the tip of the snout to the distal end of the lower maxilla; HW (head width) measured as the maximum width between temporal regions; HH (head height) taken from the interparietal scale to the gular region; NW (neck width) measured between neck flanks; SNED (snout-ear opening distance) from the tip of the snout to the anterior margin of the ear opening; SND (snout distance) from the tip of the snout to the anterior margin of the eye orbit; EED (eye-ear opening distance) from the posterior margin of the eye orbit to the anterior margin of the ear opening; AGD (axilla-groin distance) measured from the posterior margin of the insertion of the forearm to the anterior margin of the insertion of the hindlimb; BW (maximum body width) excluding pregnant females, measured between flanks at the mid-point between the forelimb and hindlimb insertions; TBW (tail-base width) measured at the tail flanks as the maximum width below the posterior margin of the cloaca; AD (arm diameter) taken from the insertion of the arm to the elbow; TD (thigh diameter) taken from the insertion of the leg to the knee; HipW (hip width) defined as the body width at the insertion of the hind legs, and TL (tail length) measured from vent to tip, and excluding specimens with damaged or regenerated tails. Measurements were taken arbitrarily on the right side of each specimen using a Mitutoyo ™ type Vernier digital caliper (± 0.01 mm). To minimize operative errors all measurements were done by the first author.

Minimum size at sexual maturity, female reproductive cycle, litter size, and mean annual reproductive output (MARO)

We determined the reproductive condition of females using morphological observations of the ovarian follicles and uteri. Follicular development was estimated by measuring ovarian follicle size (maximum diameter) in a Labklass ® stereoscopic binocular microscope with an ocular micrometer (± 0.01 mm). We characterized the uteri into three categories: Uterus type I, folds, if present, were small, and never spread over the entire uterus, similar to the thread-like oviduct typical of juveniles (*sensu* Van Wyk 1991); Uterus type II, with medium size folds spread all over the uterus, indicative of the distended oviducts of adulthood (*sensu* Vitt & Caldwell 1993), and generalized hypertrophy of the oviducts associated with vitellogenic recrudescence (Ramírez-Pinilla 1992); and Uterus type III with conspicuously more stretched oviducts than uterus type II, characteristic of recent parturition (Flemming & Van Wyk 1992, Ramírez-Pinilla 1992). Using the combination of the morphological characteristics of the reproductive tract (uteri) and follicular size (*sensu* Ibargüengoytía 2004, Boretto & Ibargüengoytía 2006, 2009, Boretto et al. 2007, Boretto et al. 2014a), we classified the females as juvenile (uterus type I and with small previtellogenic follicles), adult vitellogenic (uterus type II with enlarged vitellogenic follicles), non-reproductive adult (uterus type II and small non-vitellogenic follicles less than 4 mm), or postpartum female (uterus type III, indicative of recent parturition and small follicles less than 4 mm). Three stages of pregnancy were classified based on embryonic development (*sensu* Leyton et al. 1980), as early (from cleavage to somitic embryos), middle (curvate trunk to limbs with five fused fingers, ear and eye outlines, and abundant yolk), and late (with 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: follicles with yolk, embryos in uteri, or distended and folded uteri (Mouton & van Wyk 1993). Litter size was determined by counting the embryos in the uterus. We calculated the mean annual reproductive output as the product of litter size and litter frequency (1: annual female reproductive cycle; 0.5: biennial reproduction; *sensu* Cree 1994; 0.75: annual-biennial reproduction; *sensu* Boretto & Ibargüengoytía 2009), with the litter frequency calculated using a binomial test to analyse the differences in the proportion of pregnant (pregnant and postpartum females capture after birth dates) versus non-pregnant females (vitellogenic and non-reproductive females plus postpartum females capture after brumation period) throughout the seasons. Finally, we used two estimators of the reproductive effort of females: (1) $C = R/m$, where R is the average litter mass per female per unit of time [1 year], and m is the average mass of adult nongravid females; *sensu* Angilletta et al. 2001 and (2) an approximation of the reproductive effort in terms of the female energetic allocation, $C_{energetic} = \hat{M}_{i\text{-litters}} / \hat{M}_{i\text{-females}}$ detailed in the Supplementary Material (Tables SI, SII, SIII and SIV).

Minimum size at sexual maturity, testicular volume, and reproductive cycle of males

The minimum size at sexual maturity in males was determined based on the SVL of the smallest male with enlarged testes and convoluted epididymis (Vitt 1982). The length and width of both testicles were measured with a graduated eyepiece in a stereoscopic binocular loupe (± 0.1 mm) and the testicular volume was estimated as $V = (4/3)\pi(TL/2)(TW/2)^2$ (*sensu* Dunham 1981). To determine the spermatogenic cycle, five males were randomly selected from each season ($N_{total} = 15$) and their gonads were processed by conventional histological methods. We removed the right testis and epididymis of each specimen, dehydrated them in an ethanol series, and embedded them in paraffin. We stained the sections (7 µm) with Hematoxylin - Eosin and examined them under a stereoscopic microscope (BX40, Olympus America Inc., 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 & Wright (1970) based on the most advanced cell type present at the luminal margin of the seminiferous tubule: (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

The analyses were conducted in R statistical software, version 4.0.0 (R Core Team 2020) using the packages car, emmeans, gplots, MASS, multcompView, TukeyC, userfriendlyscience, and package 'lmodel2' (Legendre 2018) for the calculation of the b^{SMA} exponent of the scaled mass index.

For the analyses of sexual dimorphism, the variability in body measurements was explored using descriptive statistics (mean ± standard deviation, minimum and maximum) for each group of males and females. To analyze the dependence of each morphometric trait with respect to the size of the specimens, we performed simple linear regressions for each morphometric trait with the SVL. To analyze the differences between sexes, an analysis of covariance (ANCOVA) was performed using SVL as a covariate for each variable that was linearly related to SVL. For the variables that were not related to SVL, the differences between the sexes were analyzed using the t-test or Mann Whitney "U" test.

To remove the influence of body size and to evaluate the body shape differences between males and females, a second multivariate approach was performed based on the conversion of the linear morphometric characters into body proportions. We also expressed the body measurements as 13 body ratios using the largest body variables (SVL and HL) that would contain another smallest as a denominator as follows: HL/SVL; HW/SVL; HH/SVL; NW/SVL; SNED/SVL; AGD/SVL; BW/SVL; TBW/SVL; AD/SVL; TD/SVL; HipW/SVL; HW/HL; SNED/HL; SND/HL; EED/SVL. Body ratios were Arcsine Square-root transformed (Arcsin(Sqrt(i-ratio)); Sokal & Rohlf 1995). Using R we performed a Principal Component Analysis (PCA) to identify the main axes of morphometric ratio variation between sexes. PCA analyses were carried out on covariance matrices. To decide how many components could be retained, we also examined the scree plot following the "broken-stick" criterion (Peres-Neto et al. 2003). We considered the loadings as significant when their absolute values were 0.5 (Richman 1988).

To analyze the gonadal variables and fat body cycle, we performed parametric tests (t-test, ANOVA, and ANCOVA) and when the assumptions for the use of parametric statistics were not met, we used their non-parametric alternatives (Mann Whitney "U" or Wilcoxon signed-rank tests) with a confidence level of 95% and = 0.05. When significant linear relationships were found between gonadal size and SVL we used the residuals of this linear regression (ResTS) for further analyses. For the analysis of fat body weights, the variable was transformed with a natural logarithm for normalization.

RESULTS

Sexual maturity, body size, and body condition index of females

The minimum reproductive size of the females was 88 mm SVL and 29 g BM and was exhibited by a pregnant female. The sizes, weights, and body conditions of the females are shown in Table I. Adult females exhibited a significant relationship between $\hat{M}_i$ and BM (Simple Regression, $F_{1; 20} = 8.17$, $t = 2.86$, $P = 0.006$; pregnant females were excluded from this analysis). The

Table I. **Body size variables and body condition of *Phymaturus extrilidus* by sex and age classes. Mean ± standard deviation, range between parenthesis, and sample size of snout-vent length (SVL, mm), body mass (BM, g), and body condition index (scaled mass index), in BM and of pregnant females are not included.**

	Sex and Age classes			
	Females		Males	
	Juvenile	Adult	Juvenile	Adult
SVL	77.25 ± 9.48 (65.0 – 87.0) N = 9	95.05 ± 3.91 (88.0 – 103.0) N = 35	77.25 ± 4.61 (71.0 – 84.0) N = 4	95.27 ± 4.61 (85.0 – 106.0) N = 44
BM	15.74 ± 5.58 (8.25 – 28.0) N = 9	27.94 ± 4.32 (22 – 37.3) N = 21	16.25 ± 3.13 (13.2 – 26.0) N = 4	28.65 ± 4.42 (27.0 – 40.0) N = 44
M_i	26.52 ± 2.37 (23.7 – 30.1) N = 9	26.30 ± 2.87 (20.0 – 30.3) N = 21	27.54 ± 1.63 (25.9 – 29.2) N = 4	27.13 ± 3.73 (20.33 – 36.8) N = 44

SVL, however, was not significantly related to the $\hat{M}_i$ (Simple Regression, $F_{1;\,20} = 0.01$, $t = -0.313$, $P = 0.7$; pregnant females excluded from the analysis). Adult nonpregnant females did not exhibit significant differences in $\hat{M}_i$ between the seasons sampled (ANCOVA, $F_{2;\,20} = 1.41$, $P = 0.27$, BM as covariate). On the other hand, females with different reproductive states did exhibit significant differences in $\hat{M}_i$ (ANOVA, $F_{2;\,20} = 8.37$, $P = 0.002$), as vitellogenic and postpartum females showed the highest $\hat{M}_i$ than non-reproductive females (Holm-Šídák method, $t_{\text{vitellogenic-non-reproductive}} = 4.0$, $P = 0.001$; $t_{\text{postpartum-non-reproductive}} = 3.0$, $P = 0.008$; $\text{mean}_{\text{vitellogenic}} = 27.16 \pm 2.47$ g, $\text{mean}_{\text{postpartum}} = 27.20 \pm 1.43$ g; $\text{mean}_{\text{non-reproductive}} = 21.88 \pm 2.11$ g). Vitellogenic and postpartum females did not show differences in $\hat{M}_i$ ($t_{\text{vitellogenic- postpartum}} = 0.03$, $P = 0.9$).

Sexual maturity, body size, and body condition of males

In males, the minimum reproductive size was 85 mm SVL and 27 g BM and was determined in a male with enlarged testicles and convoluted epididymis. Histological analyses of testes and epididymis confirmed that males with 90 mm SVL or more, are adults. The sizes, weights, and body conditions of males are shown in Table I.

Adult males exhibited a significant relationship between SVL and $\hat{M}_i$ (Simple Regression, $F_{1;\,43} = 8.68$, $t = -2.95$, $P = 0.005$). Also, the BM showed a significant relationship with $\hat{M}_i$ (Simple Regression, $F_{1;\,43} = 9.32$, $t = 3.05$, $P = 0.003$). Adult males also exhibited significant differences in $\hat{M}_i$ between seasons (ANOVA, $F_{2;\,43} = 3.29$, $P = 0.04$). Males in early autumn had a highest $\hat{M}_i$ than in late spring (Holm-Šídák method, $t = 2.556$, $P = 0.01$; $\text{mean}_{\text{early autumn}} = 28.72 \pm 4.34$ g, $\text{mean}_{\text{late spring}} = 25.73 \pm 3.14$ g), although non-significant differences were found in $\hat{M}_i$ between early autumn and mid-spring ($t = 1.16$, $P = 0.252$; $\text{mean}_{\text{mid-spring}} = 26.88 \pm 1.80$ g), and between mid-spring and late spring ($t = 0.732$, $P = 0.4$). Adult males with different histological reproductive stages did not exhibited differences in $\hat{M}_i$ (ANOVA, $F_{3;\,14} = 2.93$, $P = 0.08$).

Sexual dimorphism

Comparison between adult males and females showed no significant differences in SVL (t-test, $t_{(78)} = -0.22$, $P = 0.8$) nor in BM (ANCOVA, $F_{1;\,64} = 3.42$; $P = 0.07$) using SVL as a significant co-variable and excluding pregnant females from this last analysis. For adult males and females we found significant relationships between HL and SVL ($F_{1; 62}$ = 7.54, t = 2.74, P = 0.007), HW and SVL ($F_{1; 62}$ = 13.02, t = 3.61, P = 0.0006), HH and SVL ($F_{1; 62}$ = 7.43, t = 2.72, P = 0.008), NW and SVL ($F_{1; 62}$ = 17.52, t = 4.18, P = 0.00001), TBW and SVL ($F_{1; 62}$ = 5.39, t = 2.32, P = 0.02), BW and SVL ($F_{1; 52}$ = 8.21, t = 2.86, P = 0.006), AD and SVL ($F_{1; 62}$ = 12.21, t = 3.49, P = 0.0008), AGD and SVL ($F_{1; 62}$ = 17.01, t = 4.12, P = 0.0001), and TL and SVL ($F_{1; 35}$ = 29.78, t = 5.46, P = 0.00001). Only TD and HipW were not related to SVL (P > 0.05). We found sexually dimorphic traits biased towards males in HL, HW, HH, TD, and TL, whereas females exhibited larger AGD than males (Table II). In the PCA analyses performed on body shape variables, 18 PCs axes were recovered, with the first three axes accounting for 69.05 % of the total variability (Table III). Hence, the retained PC1, PC2, and PC3 explained the 32.33 %, 25.40 %, and 11.32 %, respectively. The variation of TL/SVL and AGD/SVL are positively related to PC1, AGD/SVL and BW/SVL positively related to PC2, whereas the BW/SVL is negatively related to PC3 (Table III). The PCA plots with 95% confidence intervals show significant variables (Figure 2, Table III). PC1 vs. PC2 indicates a bias in the females to have higher AGD/SVL and BW/SVL, but lower TL/SVL than males. In addition, PC1 vs. PC3 BW/SVL is also significant with a bias toward females (Figure 2, Table III). The low loading values in most of the variables indicate minimal sexual dimorphism in body shape between females and males (Figure 2, Table III).

Table II. Comparison of morphometric variables between females and males of *Phymaturus extrilidus*. Mean ± standard deviation (SD), range between parenthesis, sample size (N = 27 females; N = 36 males; except N = 17 females for BW). Statistical test, F, t or U values, and degree of freedom (df). Dimorphic traits are indicated with bold and asterisk (*P* < 0.05*).

Traits	Females	Males	Significance		
	Mean ± SD	Mean ± SD	Test	Statistic value (df)	P-value
HL	16.32 ± 0.50 (15.2 – 17.1)	17.36 ± 0.84 (15.42 – 18.7)	ANCOVA	$F_{(1;60)} = 39.95$	**< 0.05***
HW	16.89 ± 0.64 (15.7 – 17.9)	18.04 ± 0.97 (16.0 – 19.7)	ANCOVA	$F_{(1;60)} = 41.65$	**< 0.05***
HH	9.99 ± 0.49 (8.8 – 11.7)	10.68 ± 0.65 (9.4 – 11.7)	ANCOVA	$F_{(1;60)} = 25.04$	**< 0.05***
NW	25.16 ± 1.26 (22.4 – 27.4)	25.36 ± 1.53 (22.1 – 27.9)	ANCOVA	$F_{(1;60)} = 0.38$	> 0.05
TBW	12.23 ± 0.72 (10.6 – 13.7)	12.36 ± 1.03 (9.8 – 14.6)	ANCOVA	$F_{(1;60)} = 0.32$	> 0.05
BW	35.49 ± 3.44 (30.4 – 42.4)	33.72 ± 3.42 (26.9 – 40.0)	ANCOVA	$F_{(1;60)} = 3.57$	> 0.05
AD	7.07 ± 0.49 (6.0 – 7.9)	7.28 ± 0.57 (6.3 – 8.3)	ANCOVA	$F_{(1;60)} = 2.60$	> 0.05
TD	8.72 ± 0.64 (8.1 – 9.9)	9.30 ± 0.66 (8.0 – 10.8)	t-test	$t_{(61)} = -3.49$	**< 0.05***
AGD	50.82 ± 3.47 (44.0 – 57.4)	49.06 ± 3.18 (42.2 – 57.3)	ANCOVA	$F_{(1;60)} = 5.52$	**< 0.05***
HipW	16.09 ± 0.89 (14.0 – 18.1)	15.96 ± 1.33 (12.4 – 20.3)	M-W U-test	U = 528	> 0.05
TL	82.27 ± 5.28 (70 – 89.5)	91.43 ± 7.58 (76 – 102)	ANCOVA	$F_{(1;34)} = 40.14$	**< 0.05***

Table III. Eigenvalues, percent of explained variance, accumulative percent of explained variance and the contributions of the variables to the factors for the first three principal components of a PCA performed on body ratios between females and males of *Phymaturus extrilidus.*

		PC1	**PC2**	**PC3**
Eigenvalue		0.002	0.001	0.0008
Percent of explained variance		32.33	25.40	11.32
Cumulated percent of explained variance		32.34	57.73	69.05
Contributions of the variables to the factors	TL/SVL	**0.797**	-0.473	-0.051
	HL/SVL	-0.045	0.118	0.254
	HW/SVL	-0.051	0.118	0.193
	HH/SVL	-0.071	0.099	0.198
	NW/SVL	0.025	0.190	0.068
	SNED/SVL	-0.048	0.023	0.060
	SND/SVL	-0.036	0.016	0.007
	EED/SVL	-0.072	0.042	0.081
	AGD/SVL	**0.556**	**0.616**	0.337
	BW/SVL	0.095	**0.500**	**-0.767**
	TBW/SVL	-0.036	0.006	0.129
	AD/SVL	-0.036	0.006	0.129
	TD/SVL	-0.037	0.125	0.131
	Wip/SVL	0.102	0.196	0.059
	HW/HL	0.008	0.006	0.139
	HH/HL	-0.071	0.099	0.198
	SND/HL	0.028	-0.079	-0.153
	EED/HL	-0.078	0.006	0.069

Follicular size and female reproductive cycle

The follicular sizes (diameter) of juvenile females varied from 1.90 to 3.14 mm (mean = 2.26 ± 0.48 mm, N = 9). Adult female follicular size was not related to SVL, BM or $\hat{M}_i$ (Simple Regression, $F_{\text{follicular size-SVL } 1;\, 20} = 0.18$, t = -0.42, P = 0.6; $F_{\text{follicular size-BM } 1;\, 19} = 1.07$, t = 1.03, P = 0.3; $F_{\text{follicular size-Mi } 1;\, 20} = 2.28$, t = 0.48, P = 0.1) excluding pregnant females. In non-reproductive adult females, the follicular size varied from 2.2 to 2.6 mm (mean = 2.33 ± 0.19 mm, N = 4). In vitellogenic females, the size of the follicles varied from 2.5 to 18.1 mm (N = 14), and were significantly different among the seasons (ANOVA, $F_{2;\, 13} = 83.45$, $P < 0.001$), particularly between mid-spring and early autumn (t = 9.23, P = 0.001) and between late spring and early autumn (Holm-Šídák method, t = 11.11, P = 0.001). No differences in follicular size were found in vitellogenic females captured in mid and late spring (t = 0.568, P = 0.58). Vitellogenesis begins in mid-spring with an increase in follicular size (mean = 3.75 ± 1.77 mm, N = 2), proceeds through late spring with slightly more enlarged follicles (mean = 4.58 ± 0.46 mm, N = 4), and concludes rapidly in autumn with mature follicles (mean = 15.98 ± 1.97 mm, N = 8; Figure 3).

Females of *P. extrilidus* exhibited different reproductive stages within and between the

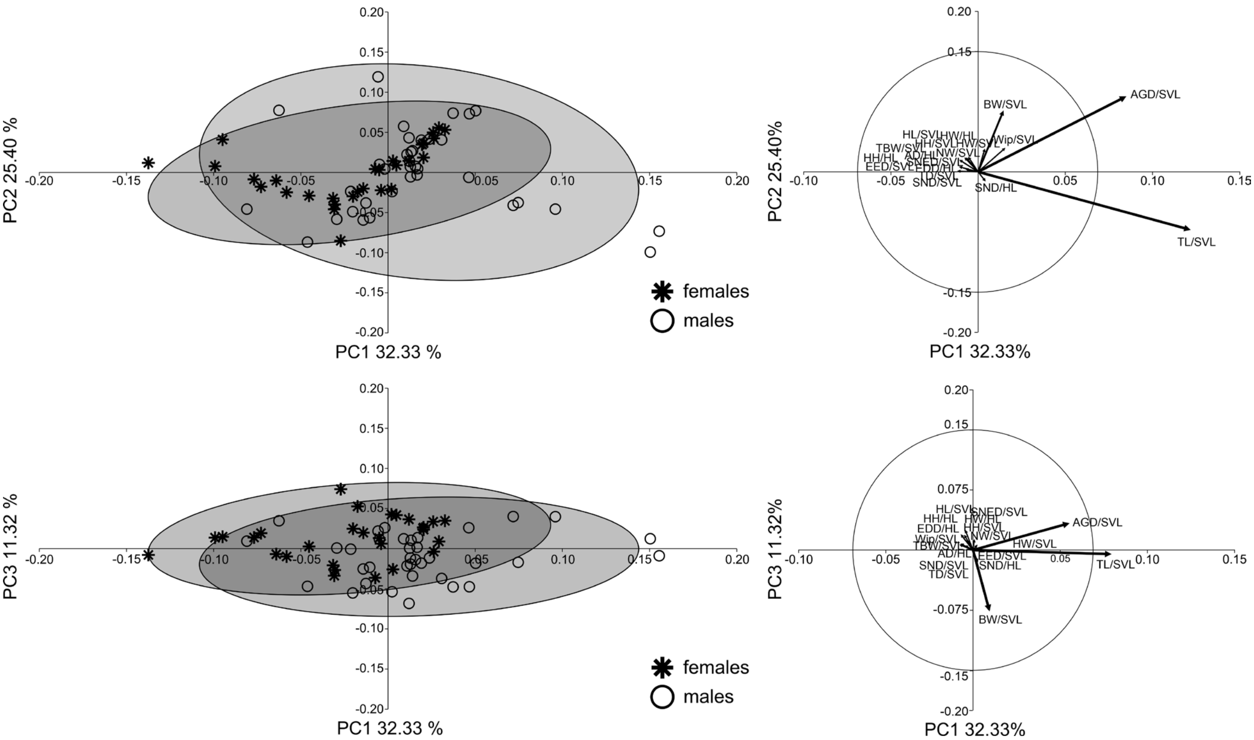

Figure 2. Results of PCA (Principal Component Analysis) on body shape variables: HL/SVL; HW/SVL; HH/SVL; NW/SVL; SNED/SVL; SND/SVL; EED/SVL; AGD/SVL; BW/SVL; TBW/SVL; AD/SVL; TD/SVL; HipW/SVL; TL/SVL; HW/HL; HH/HL; SND/HL; EED/HL between females (asterisk symbol) and males (empty dots) of *Phymaturus extrilidus*. Morphospace plotted on PC1vs. PC2 and PC1 vs. PC3, corresponding to the percentage of the total variance explained by each axis. Biplot (right panel) correspond to the contribution of each variable to the reconstruction of the axes on PC1 vs. PC2 and PC1 vs. PC3. Significant variables are indicated with thickened arrows.

three sampling seasons (Figure 4). From the total sample of adult females captured in mid-spring (October, N = 6) we found that 33.3% exhibited vitellogenic follicles (maximum diameter = 5 mm) and uterus type II, 33.3% exhibited non-vitellogenic follicles and uterus type III indicative of recent parturition, and 33.3% of females were pregnant in the initial stages of embryo development (Figure 4). From the total sample of adult females captured in late spring (December, N = 14) 28.6% exhibited vitellogenic follicles (maximum diameter = 5.6 mm) and uterus type II, 7.1% were non-reproductive (absence of vitellogenic follicles with uterus type II), and 64.3% were pregnant with embryos in medium stage development with the exception of one female with embryos in the initial stage of development. In early autumn (April, N = 15), we found that 53.3% of adult females exhibited vitellogenic follicles (maximum diameter = 18.1 mm) and uterus type II, 6.7% were post-partum females, 20.0% were non-reproductive females, and 20.0% of females were pregnant with advanced stage embryo development (Figure 4). In addition, we did not find significant differences in the ratio of pregnant (pregnant females plus postpartum females captured after birth dates) and non-pregnant females (vitellogenic females, non-reproductive females and postpartum females captured after brumation period) throughout the seasons (Proportions test, $\chi^2_{(1)} = 0.0001$, P = 1; Figure 4), indicating the biannually of the female reproductive cycle.

The SVL of the newborns varied from 48 to 50.5 mm (mean = 48.87 ± 1.18 mm, N_{newborns}

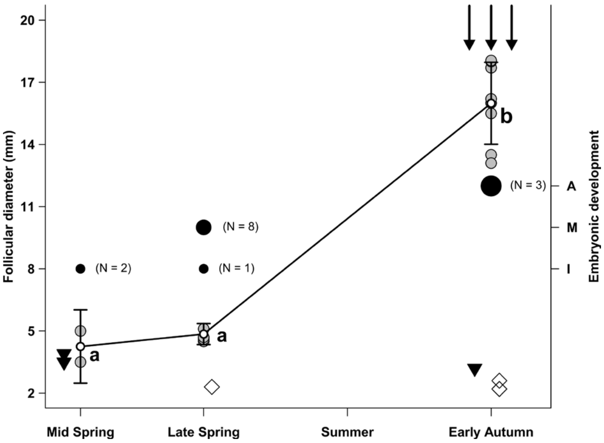

Figure 3. Follicular and embryonic development through the seasons of *Phymaturus extrilidus* females. Mean ± standard deviation of the follicular diameter of vitellogenic females is represented by empty dots and mustaches. Follicular diameter of the vitellogenic females (grey circles), post-partum females (black triangles) and non-reproductive females (white diamonds) are indicated. Occurrence of embryos with initial (I), medium (M), and advanced (A) degree of development are represented by black circles (the circles grow as embryonic development progress), and arrows indicated parturition dates (Gómez Alés, R. pers. comm.). Number of females with embryos in each stage of development are indicated between parentheses (N). The letters indicate significant differences according to *Post Hoc* test.

= 4) and the BM varied from 3.64 to 4.30 g (mean = 3.92 ± 0. 27 g). Considering the number of embryos recorded per pregnant female the litter size was 2 to 3 embryos (mean = 2.29 ± 0.47; $N_{\text{pregnant females}} = 14$), meaning that the mean annual reproductive output in *P. extrilidus* was 1.14 considering a frequency of one reproductive cycle every two years (MARO = (2.29)*1*0.5), the reproductive effort was 0.28 (C = (7.84*1) / 27.94), and the energetic reproductive effort per litter was 0.31 ($C_{\text{energetic}}$ = (7.90*1) / 25.70), detailed in the Supplementary Material (Tables SII and SIV).

Testicular size and the reproductive cycle of males

Right and left testicular volumes were not significantly different from each other (Wilcoxon signed-rank sum test, U = 1006, P = 0.96, $N_{\text{small}} = 45$, $N_{\text{big}} = 45$), so we arbitrarily choose the right testicular volume for the analyses. The testicular volume for juveniles varied from 0.80 to 3.57 mm^3 (mean = 2.24 ± 1.40 mm^3, N = 4) and from 1.87 to 181.58 mm^3 in adults (mean = 57.56 ± 41.05 mm^3, N = 44). We found a linear relationship between testicular size (volume) of adult males and the SVL ($F_{1;\,43}$ = 19.41, t = 4.405, P = 0.00001), between the ResTS and BM ($F_{1;\,43}$ = 7.252, t = 2.69, P = 0.01), and the $\hat{M}_i$ ($F_{1;\,43}$ = 9.505, t = 3.083, P = 0.003). Adult males did not exhibit significant differences in ResTS between the study seasons (ANOVA, $F_{2;\,43}$ = 1.41, P = 0.2), showing high testicular volumes in all of the activity seasons (mean $_{\text{mid-spring}}$ = 63.87 ± 22.85 mm^3; mean $_{\text{late spring}}$ = 71.30 ± 53.89 mm^3; mean $_{\text{early autumn}}$ 40.61 ± 21.71 mm^3; Figure 5).

Histological analysis reveals the progress of the spermatogenic cycle during the activity seasons (Figure 5). In mid-spring we found males with either spermatocyte (stage 2, N = 3) or in the spermatids stage (stage 3, N = 2) without spermatozoa in the epididymis. In late spring we found males with spermatozoa in the tubular lumen of the testicles and epididymis (stage 4, N = 4) or in the regression stage (stage 5, N = 1). In early autumn we found males still in spermiation (stage 4, N = 2), and others in initial gonadal regression (stage 5, N = 3), all of which had spermatozoa in epididymis, with the most abundant in males in stage 4.

Fat body storage

Adult females tended to accumulate more fat body than males, but the differences were not significant (t-test, $t_{1;\,76}$ = 1.73, P = 0.08). When pregnant females were excluded from the analysis, we found that females reserve significantly more fatty bodies than males (t-test, $t_{1;\,63}$ = 3.85, P = 0.0001; mean $_{\text{non-pregnant females}}$ = 0.18 ± 0.11 g, N = 21; mean $_{\text{pregnant females}}$ = 0.08 ± 0.06 g, N = 13; mean $_{\text{males}}$ = 0.09 ± 0.06 g, N = 43). The fat body weight of adult females was not related

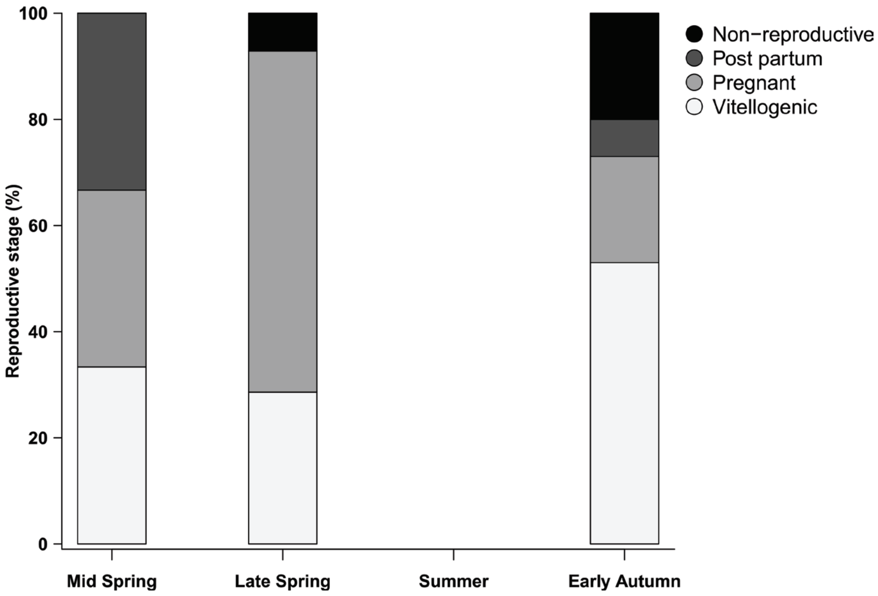

Figure 4. Percentage (%) of the reproductive stages of adult females through the seasons in *Phymaturus extrilidus* population.

to SVL, BM or $\hat{M}_i$ (Simple Regression, $F_{\text{fat body-SVL } 1;\,33} = 0.06$, t = -0.25, P = 0.8; $F_{\text{fat body-BM } 1;\,20} = 0.08$, t = 0.28, P = 0.7; $F_{\text{fat body-Mi } 1;\,20} = 0.65$, t = 0.80, P = 0.4; pregnant females being excluded from both BM and $\hat{M}_i$ analyses). Adult females did exhibit significant differences in fat body weight among seasons (ANOVA, $F_{2;\,33} = 5.97$, P = 0.006, Figure 6). In early autumn fat storage was higher than in late spring (Holm-Šídák method, t = 3.453, P = 0.002; $\text{mean}_{\text{early autumn}} = 0.20 \pm 0.11$ g, $\text{mean}_{\text{late spring}} = 0.09 \pm 0.08$). There was no significant difference in fat body weight exhibited by females captured in early autumn and mid-spring (t = 1.388, P = 0.175; $\text{mean}_{\text{mid-spring}} = 0.13 \pm 0.11$ g) nor between mid and late spring (t = 1.293; P = 0.2). We did find, however, significant differences in fat body weight between females in different reproductive stages (ANOVA, $F_{3;\,33} = 5.17$, P = 0.005), as vitellogenic females exhibited more fatty bodies than non-reproductive, postpartum, and pregnant females ($\text{mean}_{\text{vitellogenic}} = 0.21 \pm 0.11$ g, $\text{mean}_{\text{non-reproductive}} = 0.13 \pm 0.13$, $\text{mean}_{\text{postpartum}} = 0.13 \pm 0.02$ g, $\text{mean}_{\text{pregnant}} = 0.08 \pm 0.06$ g), but we only found significant differences between vitellogenic and pregnant females (Holm-Šídák method, t = 3.895, P = 0.001).

The fat body weight of adult males was not related to SVL, BM nor to $\hat{M}_i$ (Simple Regression, $F_{\text{fat body-SVL } 1;\,42} = 2.22$, t = -1.49, P = 0.1; $F_{\text{fat body-BM } 1;\,42} = 0.24$, t = -0.49, P = 0.6; $F_{\text{fat body-Mi } 1;\,42} = 0.22$, t = 0.47, P = 0.6). Adult males did exhibit significant differences in fat body weight among seasons (ANOVA, $F_{2;\,42} = 3.88$, P = 0.02, Figure 6), as fat storage was higher in early autumn than in mid-spring (Tukey test, q = 3.495, P = 0.046; $\text{mean}_{\text{early autumn}} = 0.12 \pm 0.07$ g; $\text{mean}_{\text{mid-spring}} = 0.06 \pm 0.04$ g). Males did not exhibited differences in fat body weight between early autumn and late spring (Tukey test, q = 2.997, P = 0.099; $\text{mean}_{\text{late spring}} = 0.07 \pm 0.04$ g) nor between mid and late spring (q = 1.252, P = 0.6).

DISCUSSION

Adult females of *Phymaturus extrilidus* exhibited a biennial reproductive cycle, characterized by the simultaneous occurrence of pregnant and non-pregnant females in similar proportions in all of the active seasons. Vitellogenesis begins in mid-spring and progresses through early autumn. Similarly, pregnant females were also found from mid-spring through early autumn,

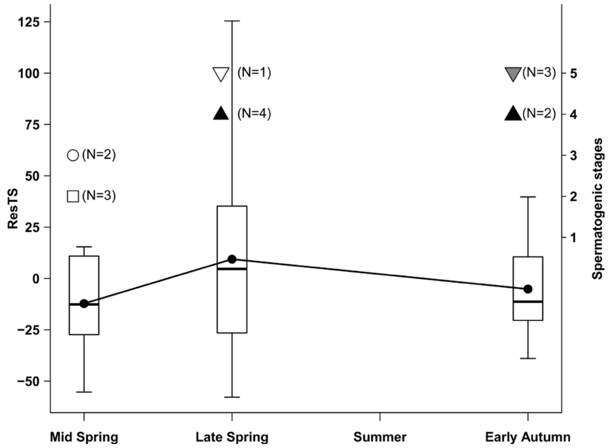

Figure 5. Seasonal variation of the residuals obtained from the lineal regression between snout-vent length and testicular volume (ResTS), and spermatogenic stages in the males of *Phymaturus extrilidus*. The plot shows the median (short-line) and the 50% of data in each season sample (box) and the whiskers are minimum and maximum values of the ResTS. Mean values of ResTS (black dots) are also represented. Spermatogenic stages found are: Stage 2 (spermatocyte, white square), Stage 3 (spermatide, empty circle), Stage 4 (spermiation, black triangle), Stage 5 (regression without spermatozoa in the epididymis: inverted empty triangle, and regression with spermatozoa in the epididymis: filled-gray inverted triangle). Sample sizes (N) are also indicated.

when births occur. The follicular cycle of female *P. extrilidus* was synchronous with the male reproductive cycle. Spermatogenesis occurs from spring to early autumn, simultaneously with the follicular development of the females, and, based on gonadal studies, we expect that mating occurs in late summer through early autumn. Males, therefore, displayed an annual and prenuptial reproductive cycle. This type of spermatogenic cycle has been observed in other species of the *P. palluma* group such as *P. vociferator* (= *P. flagellifer*, Habit & Ortiz 1996) and *P. punae* (Boretto et al. 2007), whereas *P. antofagastensis* (Boretto & Ibargüengoytía 2006) and *P. aguanegra* (= *P. cf. palluma*, Cabezas-Cartes et al. 2010) have continuous cycles. On the other hand, postnuptial spermatogenic cycles, associated with spring mating behaviors that rely on sperm stored in deferent ducts, has been described for *P. williamsi* from the *P. palluma* group (Castro et al. 2018) and for all species of the *P. patagonicus* group, such as *P. tenebrosus* (= *P. patagonicus*, Ibargüengoytía 2004), *P. zapalensis* (Boretto & Ibargüengoytía 2009) and *P. spectabilis* (Boretto et al. 2014a). In *P. extrilidus*, the presence of vitellogenic females with pre-ovulatory follicles and males with sperm in the epididymis in early autumn indicate that mating and fertilization may occur at the end of the activity season with embryonic development progressing at a reduced rate during brumation. Interestingly, we observed asynchronous gestation times during the spring months, suggesting that some males could store sperm in the epididymis during brumation and copulate with females in early spring. Future studies should be performed to confirm this hypothesis.

The vitellogenic process in female *P. extrilidus* seems to be restricted by their body energy condition. Although non-reproductive females showed a good amount of fat bodies, their body energy condition was low compared to vitellogenic and pregnant females. These results are consistent with a bioenergetic trade-off between growth and reproduction found in *P. punae* females, with vitellogenesis appearing to be more expensive than pregnancy (Boretto et al. 2015). In lecithotrophic viviparous species such as *P. extrilidus*, the yolk energy content represents the most significant component of the reproductive effort and has a higher metabolic cost than pregnancy (Van Dyke & Beaupre 2011) and, therefore, it is expected that non-reproductive females of *P. extrilidus* must reach an optimal body condition in order to begin vitellogenesis.

Prolonged biennial reproductive cycles have been observed in other *Phymaturus* species, in particular in females of the *P. palluma* group,

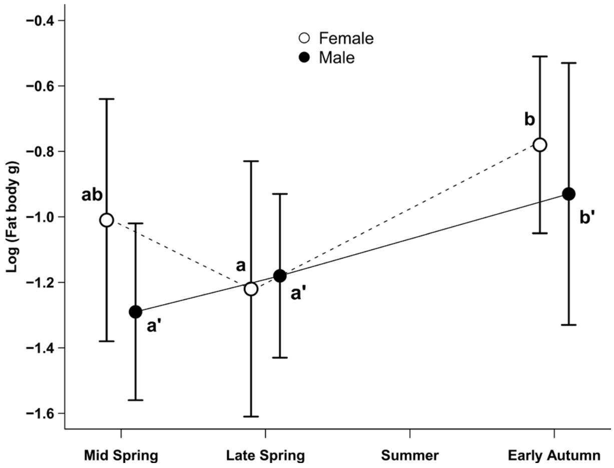

Figure 6. Seasonal variation of fat body weight [Log (Fat body g)] in adult females and males of *Phymaturus extrilidus*. Mean ± standard deviation is represented by empty dots and mustaches for females, and black dots and mustaches for males. Different lowercase letters indicate significant differences according to *Post Hoc* test.

such as *P. vociferator* (Habit & Ortiz 1996), *P. antofagastensis* (Boretto & Ibargüengoytía 2006), *P. punae* (Boretto et al. 2007), *P. aguanegra* (Cabezas-Cartes et al. 2010), and *P. williamsi* (Castro et al. 2018). The females of the *P. patagonicus* group seem to be more flexible in their reproductive timing, including biennial cycles in *P. tenebrosus* (Ibargüengoytía 2004) and annual-biennial cycles in *P. zapalensis* (Boretto & Ibargüengoytía 2009) and *P. spectabilis* (Boretto et al. 2014a). The prolonged female reproductive cycles observed in *Phymaturus* appear to be a consequence of restrictions imposed by the cold climates that the species inhabit (Cabezas-Cartes et al. 2010, Boretto et al. 2014a, Castro et al. 2018). Additionally, the duration of the cycle in *P. extrilidus* females could be conditioned by phylogeny, as has been proposed for the rest of species of the *P. palluma* group (Boretto & Ibargüengoytía 2009). Despite differences in the timing of the female reproductive cycle within the *Phymaturus* genus, births occur during mid to late summer in all of the species studied (Habit & Ortiz 1996, Ibargüengoytía 2004, Boretto & Ibargüengoytía 2006, 2009, Boretto et al. 2007, 2014a, Cabezas-Cartes et al. 2010), with the noted exceptions of *P. extrilidus*, *P. williamsi* (Castro et al. 2018), and *P. punae* (births in April, Pizarro, obs. pers.) which gave birth from late summer to early autumn.

The litter size of *P. extrilidus* was slightly different from other *Phymaturus*, as females could have two or three embryos, instead of the fixed two embryos observed in *P. tenebrosus* (Ibargüengoytía 2004) and *P. zapalensis* (Boretto & Ibargüengoytía 2009), or the variable litter size of one to two in *P. antofagastensis* (Boretto & Ibargüengoytía 2006), *P. punae* (Boretto et al. 2007), *P. aguanegra* (Cabezas-Cartes et al. 2010), and *P. williamsi* (Castro et al. 2018). An increased litter size of one to three offspring was reported for *P. spectabilis*, but only after the stressful environmental event of the Puyehue Cordón Caulle eruption (Boretto et al. 2014b). Previous to the event, the litter size was fixed at two offspring (Boretto et al. 2014a). Strikingly, female *P. extrilidus* with three embryos were always observed in spring, with the embryos in the initial stages of development. Conversely, females in advanced stages of gestation had only two embryos. Although no abortive embryos were observed in *P. extrilidus*, it has been documented for *P. antofagastesis* (Boretto & Ibargüengoytía 2006). Of all the possible processes for the abortion of embryos or eggs, extrusion seems to be the most plausible (see Blackburn et al. 2003) and is probably a consequence of a compromise between the size and number of litters regulated by the allocation of energy for reproduction (Brown 2003, Uller & Olsson 2005, Angilleta 2009, Boretto et al. 2018). For species that inhabit harsh environments, the reduction of energy loss is vital for a balanced energy allocation for both embryonic development and the mother's survival.

The trade-off between offspring size and offspring number may be affected by the limited availability of resources, resulting in either fewer offspring with larger body sizes or more offspring but with smaller body sizes (Smith & Fretwell 1974). Even if resources are unlimited, the body capacity of females can lead to a trade-off between offspring size and number, especially in viviparous species (Uller & Olsson 2005, Wang et al. 2011). In addition, large offspring with important energy reserves (high fat body contents and remnants of intra-abdominal yolk mass) can be vital to facing adverse environmental conditions when births occur just before the brumation period (Boretto et al. 2007, Boretto & Ibargüengoytía 2009, Cabezas-Cartes et al. 2010). The litter size of *P. extrilidus* was slightly larger than the other studied *Phymaturus* species, especially among species of the *P. palluma* group. Considering the litter size and the frequency of the female reproduction (one cycle every two years), the mean annual reproductive output of *P. extrilidus* was the highest recorded for species of the *Phymaturus* genus (MARO = 1.14, see Ibargüengoytía & Casalins 2007, Castro et al. 2018), though it is still considerably low. This high mean annual reproductive output is also accompanied by one of the highest reproductive efforts ($C = 0.28$ and $C_{\text{energetic}} = 0.31$) recorded for *Phymaturus*, only surpassed by *P. tenebrosus* (Boretto et al. 2018).

Since the reproductive effort is the proportion of energy that an organism allocates to reproduction over a specified and biologically meaningful time interval (Hirshfield & Tinkle 1975), the importance of standardized methods for the estimation of the reproductive investment of females led to the development of measurements that expand on the relative clutch mass index (Mesquita et al. 2016). In the present study, we proposed a variation of the reproductive effort index (C) based on the replacement of the relative litter mass by the scaled mass index of the litter, and the body mass of females by the scaled body condition of females. The scaled mass index has shown to be a powerful non-invasive tool for body condition studies based on mass-height data and shows an effective relationship with the amount of protein and fat of the body (Peig & Green 2009, 2010). In *P. extrilidus* the difference between C and $C_{\text{energetic}}$ seems to be minimal but evidence a possible subestimation of the reproductive investment of females when C is used. The $C_{\text{energetic}}$ could be a more accurate approximation of the reproductive allocation made by females due to that the scaled mass index represents the energy capital accumulated in the body as a result of feeding (Peig & Green 2009).

Different pressures or efforts in the reproductive roles are the main causes of sexual dimorphism in natural populations (Andersson 1994, Fairbairn 1997, 2007), though differences could also be due to divergences in the trophic niche to avoid competition for prey between the sexes (Cox et al. 2007, Taverne et al. 2019, Cruz-Elizalde et al. 2020). Considering the herbivorous diet of the genus *Phymaturus*, particularly strict in the *P. palluma* group (Espinoza et al. 2004, Castro et al. 2013, Boretto & Ibargüengoytía 2018), we can assume that trophic niche divergences and intrasexual or intersexual competition for prey are not the principal selection pressure on body size and shape in *P. extrilidus*. Differences in male and female body shape and size have been associated with reproductive characteristics in several lizards (Andersson 1994, Miles 2020), and this is the case for *P. extrilidus*, that showed two patterns of sexual size dimorphism (SSD). The first is male-biased, characterized by larger heads (HL, HW, HH) and more robust limbs in males that could be beneficial during social interactions, agonistic encounters or defending territories from other conspecific males (Cox et al. 2007). The other pattern is female-biased, characterized by a larger thoracic-abdominal cavity (AGD) that provides support for the hypotheses of fecundity selection (Olsson et al. 2002, Pincheira-Donoso & Hunt 2017). In *P. palluma,* as in the *P. patagonicus* group, males have larger heads, sometimes more robust limbs and neck width than females, while females have larger axilla-groin distance (AGD) and wider bodies (BW) than males (Boretto & Ibargüengoytía 2009, Cabezas-Cartes et al. 2010, Boretto et al. 2014a) as consequences of sexual selection (Olsson et al. 2002, Cox et al. 2007) and fecundity selection (Andersson 1994, Olsson et al. 2002, Cox et al. 2007), respectively.

Considering body shape, the results showed that females exhibit larger sizes in AGD/SVL and BW/SVL, but smaller in TL/SVL, and these traits may be explained by a combination of both fecundity and natural selection (Schwarzkopf 2005, Scharf & Meiri 2013). As noted earlier, large thoracic-abdominal cavities and body width are associated with offspring size and number (Olsson et al. 2002), but also have been related to differential foraging and predator escape strategies during pregnancy, suggesting that clutch volume and body shape should coevolve to maximize reproductive success, minimizing the mortality of pregnant females and enhancing the survival of juveniles (Vitt & Congdon 1978, Vitt 1981, Werneck et al. 2009). In addition, the tail is the main lipid reserve structure in some liolaemid lizards (Paz et al. 2019), and an eventual tail loss could negatively impact the reproductive cycle (Maginnis 2006). Since reproduction in *Phymaturus* females represents a high energy demand in the form of lipids during vitellogenesis, the observed differences in the tail shape between males and females of *P. extrilidus* could be a strategy to avoid tail loss. Relatively shorter tails in females could maximize the possibility of escape from a predator attack without losing their tail (Amo et al. 2007, Cooper & Blumstein 2015) or minimize the chance of tail loss during intraspecific aggression (Pafilis et al. 2009) as it may directly affect the individual´s fecundity (Dial & Fitzpatrick 1981, Jennings & Thompson 1999).

The morphological differences found between males and females of *P. extrilidus* could be the result of pressure from sexual selection, given the fact that, when males are in spermiation during the mating season, the proportion of receptive females is approximately half of the female population, as the other half is pregnant, postpartum, or non-reproductive. This reproductive population biased towards males could increase competition and intensify sexual selection (Sugg et al. 1995). A male-biased operational sex ratio (OSR) has been confirmed in *P. tenebrosus* (Ibargüengoytía 2004), *P. antofagastensis* (Boretto et al. 2007), *P. zapalensis* (Boretto & Ibargüengoytía 2009), *P. aguanegra* (Cabezas-Cartes et al. 2010), and *P. spectabilis* (Boretto et al. 2014a) and could be possibly present in *P. extrilidus*, though new population studies are necessary to confirm this hypothesis. In addition, we found that the bigger males exhibited the lowest body conditions, probably as a consequence of the energy allocation in intrasexual competition for females, mating behaviors, and territory defense. Similarly, among the species of the *P. palluma* group, the strictly biennial reproductive cycles of females also lower the number of females that are available at the time of mating and therefore increase sexual selection pressure in males. Among species of the *P. patagonicus* group, however, the annual-biannual cycles decrease sexual selection pressure, but increase the fecundity selection pressure, resulting in larger females than males (review in Boretto et al. 2020). Valdecantos et al. (2019) added an external factor to the discussion of sexual dimorphism as they discovered that females of the genus *Phymaturus* tend to be larger than males when the mean air temperature during the activity season decreases, suggesting that climate may be another significant factor in the fecundity hypothesis.

Present study describes relevant life-history traits related to the reproductive biology of *P. extrilidus*, a poorly studied species endemic to the Sierra de la Invernada in San Juan province at more than 3000 m asl. In this species, and in all the *Phymaturus* genus, the viviparous reproductive mode has developed as an adaptation to cold environments, as suggested by the cold climate hypothesis (Shine 1995, Pincheira-Donoso et al. 2013, Mesquita et al. 2016, Esquerré et al. 2019). However, the costs associated with viviparity, several found in the present study of *P. extrilidus*, such as the small litter sizes, high reproductive investment of females to provide energy reserves to their offspring, long interbirth intervals, and low body condition in post-partum females, among others, highlights the high parental investment required for survival of offspring's in constraining environments, increasing the vulnerability of the species.

Acknowledgments

We dedicate this work to Andrés Calderón who passed away after the end of the field work. Special thanks to Arturo Curatola for encouraging our research and for the logistical support in the Don Carmelo reserve. We thank Ezequiel Salomón and Yanina Ripoll for providing the collection permit. Specimens were collected under permission of Secretaría de Medio Ambiente and Dirección de Conservación y Áreas Protegidas, Provincia de San Juan, Argentina (Exp. N° 1300-4047-12). Special thanks to Ana Naranjo (EUCS - UNSJ) for the constant support and for encouraging our research. We thank Jennifer Richardson for language assistance and improvements in the manuscript. We also thank CIMA/CONICET-UBA for providing climatic data of the 3ra. Comunicación Nacional sobre Cambio Climático. Financial support was received from Universidad Nacional de San Juan granted to AL (CICITCA E/1101) and Fondo para la Investigación Científica y Tecnológica (FONCYT PICT-2017-0905) to JMB. Finally, we also acknowledge the helpful comments of three anonymous reviewers.

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SUPPLEMENTARY MATERIAL

Tables SI-SIV.

Appendix I. Examined specimens

Phymaturus extrilidus (N = 96): ARGENTINA: San Juan province: Ullum department: Sierra de la Invernada: The Don Carmelo Reserve of Multiple use (30° 56′ 59″ S, 69° 04′ 50″ W; 3100 m asl). April 2014: UNSJ 1943 - UNSJ 1984 (42 specimens: 19 females, 19 males, and 4 newborns). December 2014: UNSJ 2259 - UNSJ 2296 (40 specimens: 18 females, and 22 males). October 2016: UNSJ 2645 - UNSJ 2658 (14 specimens: 7 females and, 7 males).

How to cite

PIZARRO JE, LASPIUR A, ACOSTA JC, BLANCO GM & BORETTO JM. 2022. High reproductive effort in a vulnerable lizard from high altitudes in Argentina: Reproductive biology and sexual dimorphism in *Phymaturus extrilidus*. An Acad Bras Cienc 94: e20210179. DOI 10.1590/0001-3765202220210179.

Manuscript received on February 05, 2021;
accepted for publication on October 11, 2021

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Author contributions

Jesús E. Pizarro designed the study, participated in the sampling and analysis of samples, data acquisition and curation, methodology, results analysis, wrote the original draft and final version of the manuscript, and designed and constructed the figures. Alejandro Laspiur participated in the sampling and analysis of samples, methodology, results analysis, wrote the original draft and final version of the manuscript, designed and constructed the figures, and funding acquisition. Juan C. Acosta and Graciela M. Blanco designed the study and methodology and contributed to the original draft and final version of the manuscript. Jorgelina M. Boretto designed the study, participated in the analysis of samples, methodology, results analysis, wrote the original draft and final version of the manuscript, designed and constructed the figures, and funding acquisition. All authors discussed the results and commented on the manuscript.