Energy allocation to growth and reproduction in a viviparous lizard endemic to the highlands of the Andes, Argentina

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Abstract

Individuals devote energy intake to growth, maintenance and reproduction, so knowing the way in which they allocate this energy between competing demands is essential to understand their relationship with the environment. *Phymaturus* lizards, viviparous and mostly herbivorous, inhabit cold environments of the Andes highlands of Argentina and Chile and the volcanic plateaus of Patagonia, Argentina. Herein, we discussed the interplay between reproductive effort and growth in *Phymaturus punae*, a lizard with a biannual female reproductive cycle and the lowest annual reproductive output of the genus. Using skeletochronology, we estimated that juveniles reach up to 7–8 years and that adults have 8–18 years in females and 9–20 years in males. Juveniles grow faster than adults and adult females have higher specific growth rates than adult males. The adult life span was 10 years for females and 11 for males and the relative reproductive time was 1.25 for females and 1.22 for males. Net reproductive rate resulted in 7.5 offspring throughout the females' reproductive life. During adulthood a negative correlation between the ring width and age was found in males, while in females the ring width showed a pattern related with the yearly reproductive state. Females tend to have wider rings when pregnant and narrower rings when vitellogenic. The intersexual differential investment in growth is congruent with different requirements for reproduction between genders, whereas the tendency of females to grow less during vitellogenesis than during pregnancy, suggests differences in the metabolic costs of these reproductive processes. Even when *P. punae* exhibits high life span, females have a relative low lifetime reproductive effort and net reproductive rate. However, this still represents a high reproductive investment for this species, considering the harsh conditions of the environments where it lives, the viviparous reproductive mode and their herbivorous diet.

Introduction

The ambient temperature plays a critical role in determining the life-history patterns of ectotherms, considering its influence on biological rates and, in some cases, on the trajectories of biochemical reactions that affect vital parameters of the whole organism (Adolph & Porter, 1993; Zug, Vitt & Caldwell, 2001). The rates of biochemical reactions decline with decreased body temperature (Zug *et al.*, 2001), producing a physiological time delay relative to chronological time: life cycles are longer, sexual maturation occurs later and there is higher longevity (Shuter & Post, 1990). Under this paradigm in environments with cold climates, species afford longer periods of trophic deficiency (Shuter & Post, 1990), have short periods of activity and the best time for mating may not be the best time for birth (Pough *et al.*, 1998). In this sense, the reproductive mode (Tinkle & Gibbons, 1977), the relative time of reproductive

events (Saint Girons, 1985) and the time at which births occur to favor the survival of newborns (Tinkle & Gibbons, 1977; Pough *et al.*, 1998) are of vital importance in reptiles.

The lizard genus *Phymaturus*, viviparous and mostly herbivorous, inhabits cold environments of Andean highlands of Argentina and Chile and the volcanic plateaus of Patagonia, Argentina (Cei, 1986). *Phymaturus* females have shown prolonged biannual reproductive cycles (one litter every 2 years; Habit & Ortiz, 1996; Ibargüengoytía, 2004; Boretto & Ibargüengoytía, 2006; Boretto *et al.*, 2007; Cabezas-Cartes *et al.*, 2010) or annual/biannual characterized by skipping a reproductive season (Boretto & Ibargüengoytía, 2009; Boretto *et al.*, 2014), as consequence of short activity seasons and limited opportunities for thermoregulation in their cold environments. Additionally, *Phymaturus* have litter sizes of one to two offspring and fecundity rates are among the lowest for lizards (Ibargüengoytía & Casalins, 2007).

In a limiting environment, it is expected that *Phymaturus* lizards will present an energy trade-off among growth, maintenance and reproduction. We have evidence of the environmental restraints on *Phymaturus* reproduction, but there is only one study on growth dynamics performed in *P. tenebrosus* (Piantoni, Ibargüengoytía & Cussac, 2006a). This species shows late sexual maturity (*sensu* Tinkle, Wilbur & Tinlley, 1970; 7 years in females and 9 years in males) and lives up to 16 years (Piantoni et al., 2006a). Late sexual maturity allows females to reach sexual maturity at large body sizes and to give birth to larger offspring (Piantoni et al., 2006a). As in *P. tenebrosus*, births occurs at the end of summer and hibernation starts in early autumn (Ibargüengoytía, 2004), larger offspring sizes are expected to increase over winter survival (Piantoni et al., 2006a).

The proportion in which energy is destined during ontogeny depends on sex, sexual maturity, reproductive condition, food availability and predation pressure and abiotic factors such as temperature, photoperiod and the extending of the active season (Pianka, 1982; Stearns, 2000; Niewiaroswski, 2001). Juveniles often have higher growth rates than adults and earlier sexual maturity when resources are relatively abundant (Niewiaroswski, 2001). In adults, the allocation of time, energy and materials for reproduction and brood care (reproductive effort, Stearns, 2000; Charnov, 2005) decreases the growth of somatic tissues and often reduces future fecundity (Pianka, 1982; Stearns, 2000). The reproductive effort is measured as the fraction of body mass expended in reproduction per year, so involves a trade-off with other needs of the individual, as investment in growth and maintenance (Stearns, 2000; Charnov, 2002), and is inversely proportional to average adult life span (Charnov, 2005). In most reptiles, there is a strong positive relationship between growth and fecundity because the volume of the abdominal cavity and hip size limit reproductive capacity in number and size of offspring or eggs (Chamaillé et al., 2006). In *Phymaturus*, females have higher interlimb length than males, probably to increase the body space available to shelter the developing embryos (Boretto & Ibargüengoytía, 2009; Cabezas-Cartes et al., 2010; Boretto et al., 2014).

The latitudinal and altitudinal conditions in which *Phymaturus* species inhabit, their particularly prolonged female reproductive cycle and the low annual reproductive output make them a unique model to study the energetic trade-offs in cold environments. Skeletochronology, based on the count of growth marks deposited in bones, has become a reliable and powerful tool to assess biological time, representing a standard method for individual age estimation in vertebrates (e.g. Leclair & Castanet, 1987; Castanet & Smirina, 1990; Castanet et al., 1993). The individual age and growth rates of Liolaemids have been studied in lizards from Patagonia (*P. tenebrosus*, Piantoni et al., 2006a; *Homonota darwini*, Piantoni, Ibargüengoytía & Cussac, 2006b; Kubisch et al., 2012; *Liolaemus pictus argentinus*, Gutiérrez, Piantoni & Ibargüengoytía, 2013) and from high altitudes in the north of Argentina (*Liolaemus irregularis*, *Liolaemus multicolor*, Valdecantos, Lobo & Martínez, 2007). Herein we study growth rate, life span, age at sexual maturity, life time for

reproduction and reproductive effort of *P. punae* that inhabit in harsh and cold environments at high altitudes (3500–4200 m asl) in the Andes Mountains. We test the hypotheses that *P. punae* (1) exhibit slow growth rates, late sexual maturity and extended lifetime span because of its cool environment; (2) growth parameters vary during ontogeny and by sex; (3) males and females exhibit differences in the annual growth rate (thickness of each ring) related to differences in reproductive investment; (4) adult females exhibit differences in annual growth according to the reproductive event held each year (pregnancy vs. vitellogenesis); (5) has high reproductive effort and relative reproductive investment, low rates of net reproduction and low reproductive values. We discuss our results comparing with other liolaemids studied up to today.

Materials and methods

Specimens

We analyzed two offspring, four juveniles, nine adult females and 12 adult males of *P. punae* from the collection of the Department of Biology and Institute and Museum of Natural Science, Universidad Nacional de San Juan. These individuals were collected in spring (December) 2004 and summer (February) 2005, in the Provincial Reserve San Guillermo, San Juan province (Argentina, 28°59′ to 29°02′S; 69°29′ to 69°05′W, 3100–4200 m asl). The specimens of *P. punae* examined correspond to the entire collection and the sample was necessarily small because of the protected conservation status of the species that inhabit the Biosphere Reserve Park of the Park San Guillermo, its endemism in this site and its vulnerable conservation status (Abdala et al., 2012) that exclude the possibility of capturing more lizards.

Environment characteristics

The Provincial Reserve San Guillermo extends over 170 000 hectares (Salvioli, 2007), has a desert cold climate with low temperatures throughout the year and low precipitations (Fig. 1), especially in the highlands regions of the Reserve, above 3000 m asl. In winter, maximum winds speed from the Pacific Ocean overpass the 120 km h^{-1} and between 3000 and 6000 m asl, precipitations were mostly as snow, hail and glitter (Salvioli, 2007). The phytogeographic region is characterized by Nanophanerophytas as *Larrea nitida*, *Larrea divaricata*, *Bulnesia retamo*, *Lycium*, *Adesmia* and *Senecio* and rockrose xerophytes and the grass family (Gramineae) as *Stipa* (Cajal, Reca & Pujante, 1981).

Morphometrical variables and reproductive data

Data of snout-vent length (SVL, digital gauge ± 0.02 mm, CA-01, Lee Tools, Guangzhou, Guangdong, China), sex and body mass (100 g spring scale ± 0.5 g; Pesola AG, Baar, Switzerland) of each specimen were registered. The minimum SVL at sexual maturity and the reproductive stages of the
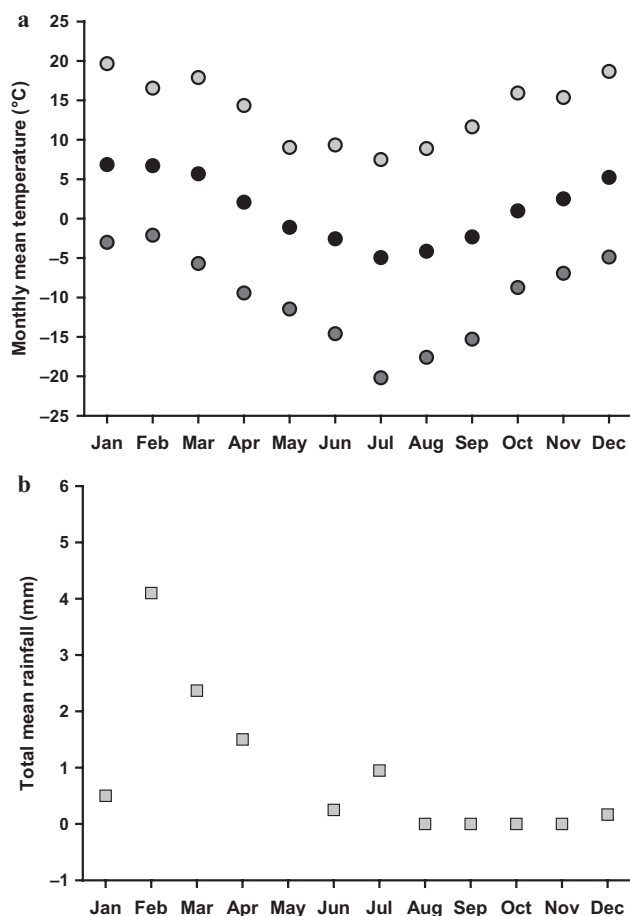
Figure 1 Climatic data from the nearest meteorological station to the study site provided by the Secretaría de Minería of the Government of San Juan (3800 m asl). (a) Monthly means of maximum (gray circle), minimum (dark gray circle) and mean (black circle) temperatures (°C) and (b) monthly means of total precipitation (mm) from 1998 to 2003 are presented.

individuals were determined in Boretto *et al*. (2007), based on morphological and histological analysis of gonads.

Histological technique

Arbitrarily, the right femur of each specimen was removed and left in 7% nitric acid from 2 to 7 h for decalcification; then the femur was dehydrated through a series of increasing concentrations of ethanol solutions, cleared with toluene and embedded in paraplast for 24 h at 60°C. Cross-sections at mid-diaphyseal level were stained with hematoxylin-eosin (Martoja & Martoja Pierson, 1970).

Determination of growth rate, age and longevity

The histological preparations were analyzed under an optic microscope (Olympus BX40, America Inc., Melville, NY,

USA) equipped with a Pro-Series High Performance CCD camera. Digital images were measured using an Image-Pro Plus analyzer (Media Cybernetics, Inc., Rockville, MD, USA). To avoid under- or overestimating the age of lizards, the presence of endosteal bone and the first bone zone (next to the medullar cavity or to the endosteal bone) were considered. The 10 best sections of each bone were selected to estimate (Leclair & Castanet, 1987): (1) minimum and maximum radius from the center of the medullar cavity; (2) minimum and maximum diaphyseal diameter; (3) estival layers or ring thickness; (4) number of lines of arrested growth (LAGs). The average of the minimum and maximum radius of each variable was calculated to minimize the asymmetry of the bone sections and medullar cavity.

When endosteal resorption occurred, the estimated age was calculated using the size of the first growth marks of offspring in order to back-calculate the number of rings removed (Piantoni *et al*., 2006a,b; Gutiérrez *et al*., 2013). The number of reabsorbed rings was added to the number of observed rings. To determine the relationship between individual SVL and age, the best fitted curves using the highest r^2 were selected, using Table Curve 2D Version 5.01 (SYSTAT Software Inc., 2002). Then, we calculated the corrected Akaike information criterion (AICc) of the best models to decide which growth model provided the best fit (Angilletta, 2006). Growth rates were calculated as the derivative of the curve of SVL versus age. Specific growth rates were obtained by dividing the growth rates by the SVL of each specimen.

Width of bone zone

We measured the ring widths to analyze the variation in bone growth between adult males and females, and between females that the last year performed vitellogenesis versus females that the last year performed gestation (considering their reproductive state studied in Boretto *et al*., 2007). The most external zone which corresponded to the year of capture was removed from the analysis to avoid errors arising from incomplete growth, as captures were in late spring and in midsummer.

Relationship between growth and reproduction

We used data from the present study and from Boretto *et al*. (2007) as: (1) period of time between age at maturity and maximum longevity for males and females; (2) clutch size; (3) frequency of female reproduction; (4) offspring mass and (5) post-partum mass of females to estimate dimensionless numbers (Charnov, 2002, 2005), each one representing a benefit-cost ratio summarizing reproductive timing (*relative reproductive time*, $T = E/\alpha$, where E is the average adult life span, α is age at first reproduction) and reproductive effort (*reproductive effort*, $C = R/m$, where R is the average of offspring mass per unit of time and m is the average adult mass). We calculated the lifetime reproductive effort ($LRE = (R \times E)/m$) and the net reproductive rate (R_0) as the average number of offspring produced over a mother's

lifetime (*sensu* Charnov, 2002, 2005; Charnov, Warne & Moses, 2007). For comparative purposes, we calculated the life-history traits for *P. tenebrosus* using published data (Ibargüengoytía, 2004; Piantoni *et al.*, 2006*a*).

Statistical analysis

We used the statistical software Sigma Stat 3.5® (Systat Software Inc., Chicago, IL, USA) and Sigma Plot 10.0® (Systat Software Inc.). We used analysis of linear regression, Pearson's correlation, *t*-test, Mann–Whitney and Wilcoxon test. Assumptions of normality and homogeneity of variance were tested with the one-sample Kolmogorov–Smirnov test and with the Levene test, respectively. Means are given ± SE.

Results

Bone histology and bone growth patterns

The femur diaphysis of *Phymaturus punae* showed a layer of periosteal bone with numerous osteocytes and a medullar cavity larger in diameter in older individuals. The periosteal bone exhibited LAGs as stripes strongly stained by hematoxylin, following the ellipsoidal bone shape. LAGs, which correspond to the winter period, appeared between lighter and thicker zone, which correspond to summer growth period (Fig. 2). LAGs were absent in offsprings born in captivity ($n = 2$; $SVL_{range} = 50.9$–51.3 mm; Fig. 2a), but the rest of the specimens showed up to 13 LAGs ($n = 25$; $SVL_{range} = 78.2$–104.4 mm; Table 1; Fig. 2b–d).
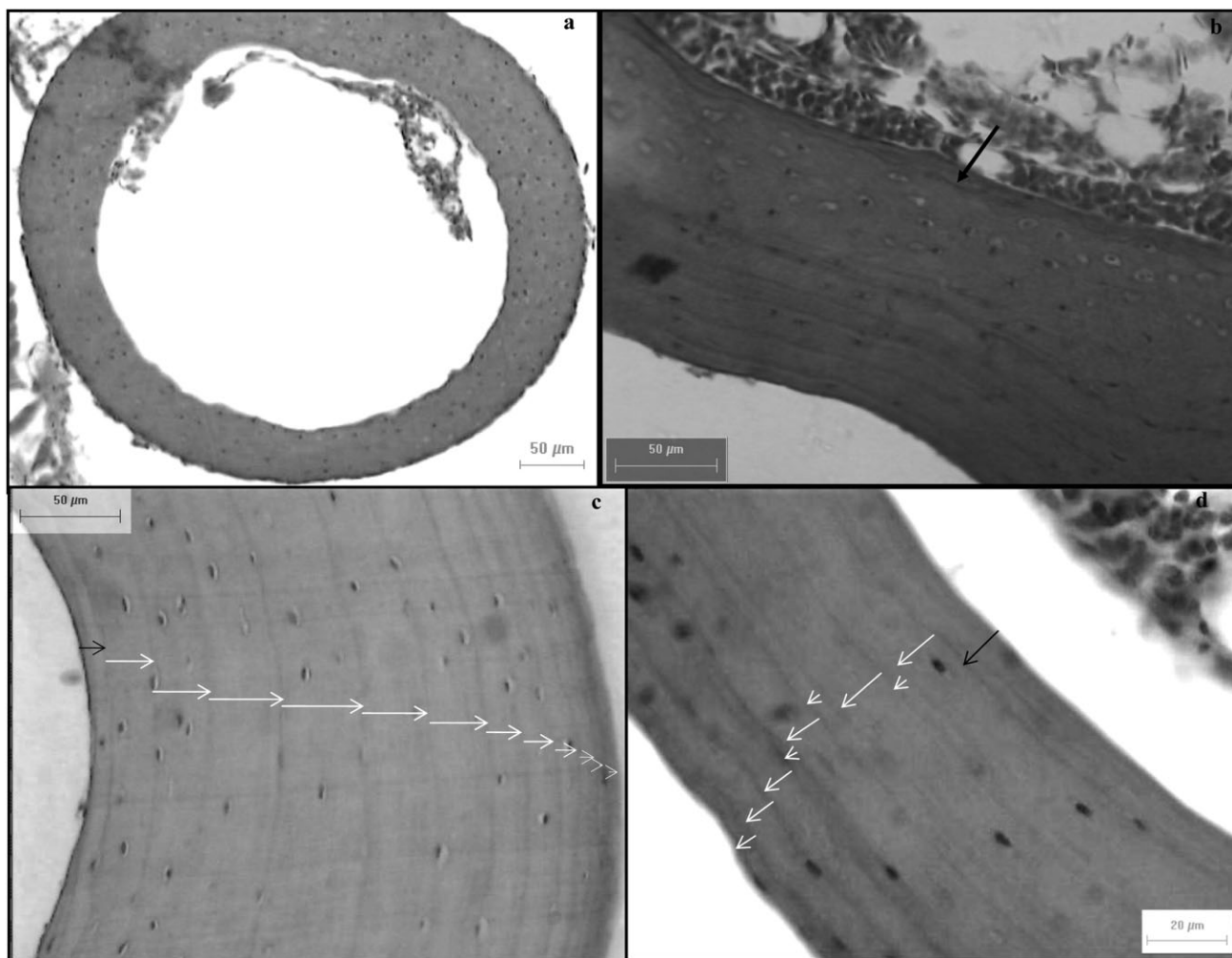

Figure 2 Diaphysial cross-section of different individuals of *Phymaturus punae*: (a) newborn showing a single ring at birth (×100); (b) adult specimen showing endosteal bone and line of resorption, proximal to the medullar cavity (black arrow; ×200); (c) adult male showing endosteal bone and line of resorption (black arrow) and 12 lines of arrested growth (LAGs; white arrows; ×200); (d) adult female showing endosteal bone, line of resorption (black arrow), alternation of wider and narrower rings and nine LAGs (white arrows; ×400).

Table 1 Comparison among newborns, juveniles, adult females and adult males of *Phymaturus punae*

|	Newborns (*n* = 2)	Juveniles (*n* = 4)	Males (*n* = 12)	Females (*n* = 9)	Total adults (*n* = 21)
Snout-vent length (mean ± SE; range; mm)	51.08 ± 0.18; 50.90–51.26	80.50 ± 1.04; 78.16–82.38	95.37 ± 1.68; 84.40–104.42	89.44 ± 1.67; 80.64–95.30	92.83 ± 1.34; 80.64–104.42
Counted rings	0	4–7	7–13	7–13	7–13
Number of reabsorbed rings	–	1–2	2–8	1–5	1–8
Estimated age (mean ± SE; range; years)	0	6.50 ± 0.87; 5–9	15.58 ± 0.79; 9–20	13.00 ± 0.85; 9–18	14.48 ± 0.64; 9–20
Specific growth rate (median; range; [mm × (year × mm)⁻¹])	–	0.0297; 0.0223–0.0335	0.0132; 0.00976–0.0218	0.0160; 0.0120–0.0228	0.0140; 0.00976–0.0228
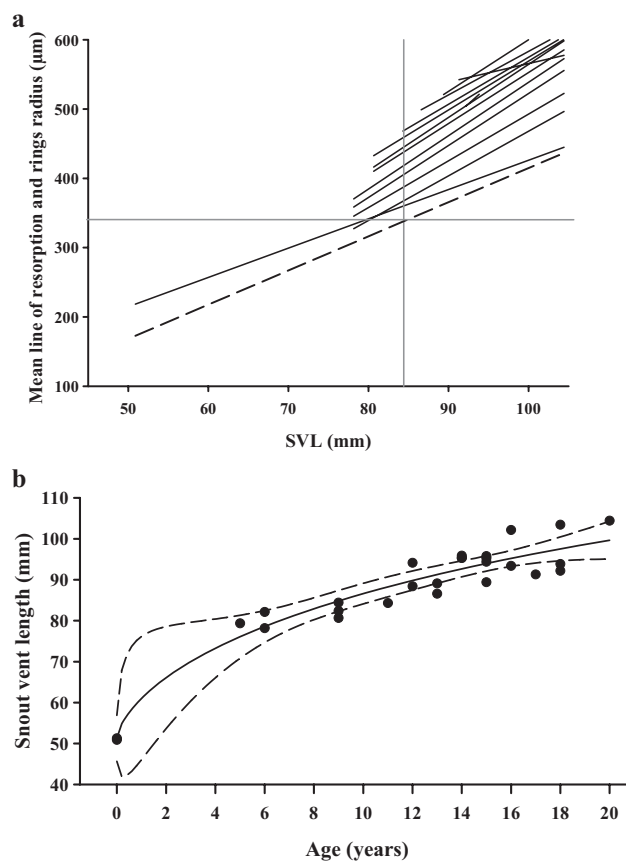
Figure 3 (a) Estimation of medullar resorption in *Phymaturus punae*. Regression lines of the line of resorption radius versus snout-vent length (SVL; long dashed line) and of the radius of LAGs versus SVL (continuous lines) are presented. Medullar resorption was calculated placing a vertical line (gray) on a particular SVL and a horizontal line (gray) on the intersection between the vertical line and the line of resorption radius regression line. The number of reabsorbed rings at a certain SVL corresponds to the number of regression lines that are under the horizontal line placed for that size (*sensu* Piantoni *et al.*, 2006*a,b*; Gutiérrez *et al.*, 2013). (b) Logistic function fitted for the relationship between SVL and age in *P. punae*.

Bone resorption and estimated individual age

Mean medullar radius and mean resorption line radius incremented with SVL (linear regression, $F_{\text{medullar radius1, 27}} = 39.304$, $r^2 = 0.611$, $P < 0.001$; $F_{\text{resorption line radius1, 24}} = 13.803$, $r^2 = 0.386$, $P < 0.001$). Medullar resorption removed from one to two rings in juveniles ($n = 4$) and from one to eight rings in adults ($n = 21$; Fig. 3a; Table 1). The estimated age, adjusted according to reabsorbed rings, ranged from 6 to 9 years old in juveniles, from 9 to 18 years old in adult females and from 9 to 20 years old in adult males. The logistic function LgstcDoseRsp ($F_{3,26} = 87.779$, $r^2 = 0.919$, $P < 0.0001$; AICc = 32.592; Fig. 3b) was the best fitted model to show the relationship between the
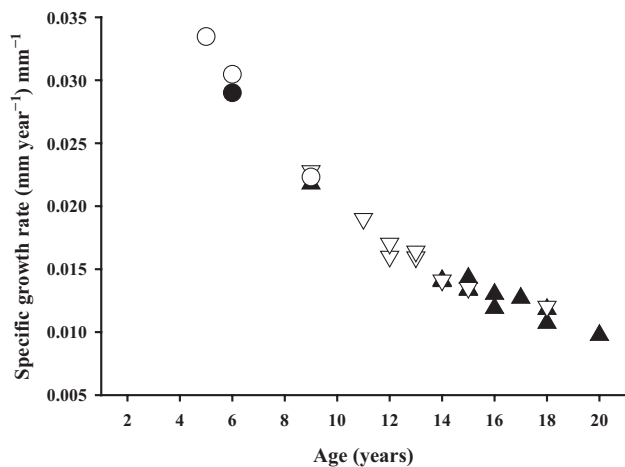
Figure 4 Specific growth rates of *Phymaturus punae* juvenile males (black circles) and females (white circles), adult females (white triangles down) and adult males (black triangles up) versus age.

estimated age and SVL, according to r^2 of 488 iterations and AICc.

Growth rates and reproductive life-history traits

Juveniles have higher specific growth rates than adults (*t*-test, $t = 7.322$, d.f. $= 23$, $P < 0.001$) and adult females have higher specific growth rates than adult males (Mann–Whitney, $T = 133.50$, $P = 0.016$, $n = 21$; Fig. 4; Table 1). The youngest adult female presented 9 years old and was pregnant. But, we consider that this female has achieved sexual maturity the previous year, at 8 years old, when it started the vitellogenesis according to our former study of reproductive biology, which showed that *P. punae* females devoted an entire activity season (late spring to early autumn) for vitellogenesis and the next for pregnancy (biennial reproductive cycle, Boretto *et al*., 2007). The reconstruction of the reproductive history of each female studied (*n* = 9) showed that six adult females were pregnant at the age of 9 years old (Fig. 5a,b), adding support to the acquisition of sexual maturity at 8 years old when they were vitellogenic. Sexual maturity was found to be reached at 9 years old in males. The maximum age was 18 years for females and 20 years for males.

Considering the youngest adult and the maximum age registered, the adult life span (E) was 10 years for females and 11 years for males and the relative reproductive time (T) was 1.25 for females and 1.22 for males. As female reproductive cycle is biannual and the clutch size is one to two offspring, the mean annual reproductive output is 0.75 (Boretto *et al*., 2007) and the net reproductive rate (R_0) resulted in 7.5 offspring along the entire reproductive life of each female. The relative clutch mass was 0.41, the reproductive effort was 0.100 and the lifetime reproductive effort (LRE) was 1.05. For *P. tenebrosus*, we estimated an E of 9 years for females and 7 years for males, considering the youngest adult was 7 years for females and
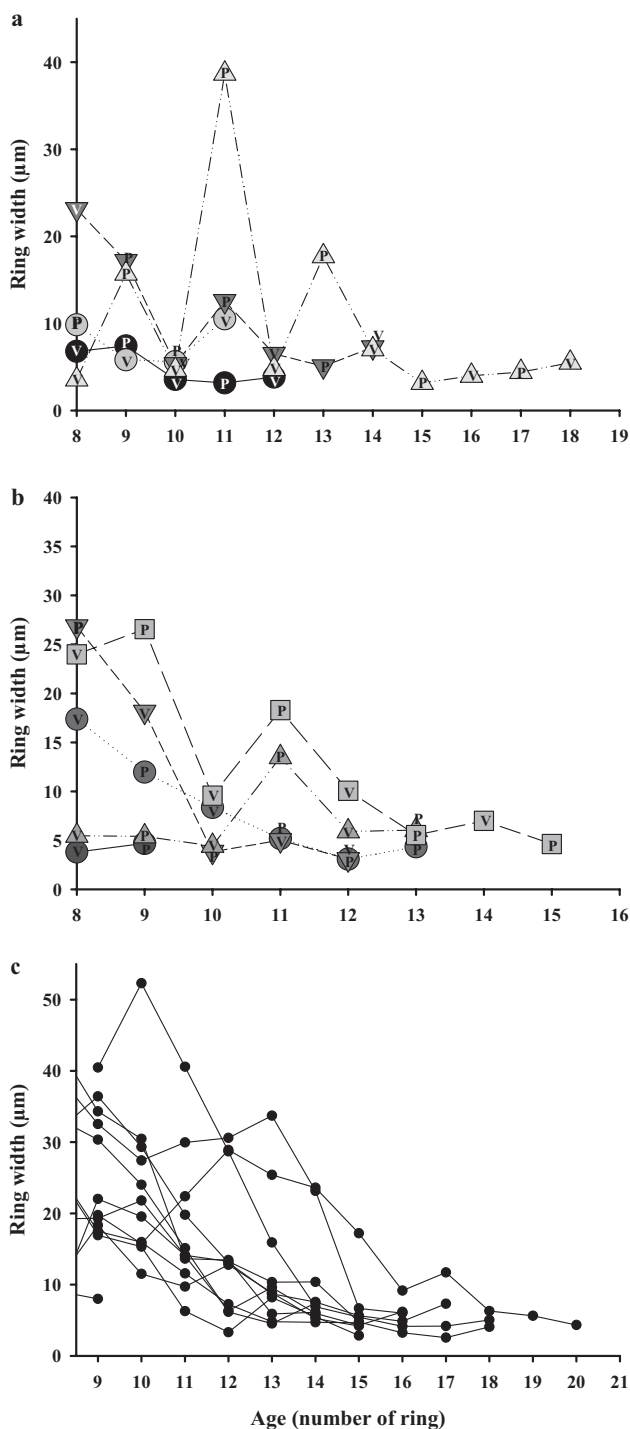
Figure 5 Rings width of adult females of *Phymaturus punae* that were captured performing vitellogenesis (a) or pregnancy (b), indicating the reproductive stage developed each year (vitellogenesis: V; pregnancy: P). Each type of line represent a different female. (c) Rings width of males after sexual maturity.

Table 2 Relationship between the number of ring and the ring width of each adult specimen of *Phymaturus punae*

Sex	Snout-vent length (mm)	All rings of each specimen	After sexual maturity
Male	84.4	$r = -0.752$; $P = 0.0849$; $n = 6$	–
Male	95.86	$r = -0.513$; $P = 0.194$; $n = 8$	$r = -0.957$; $P = 0.0107$; $n = 5$[a]
Male	95.3	$r = -0.791$; $P = 0.0195$; $n = 8$[a]	$r = -0.983$; $P = 0.0025$; $n = 5$[a]
Male	95.76	$r = -0.761$; $P = 0.0171$; $n = 9$[a]	$r = -0.856$; $P = 0.0297$; $n = 6$[a]
Male	95.24	$r = -0.818$; $P = 0.00706$; $n = 9$[a]	$r = -0.782$; $P = 0.0664$; $n = 6$
Male	89.38	$r = -0.937$; $P = 0.0000656$; $n = 10$[a]	$r = -0.973$; $P = 0.00109$; $n = 6$[a]
Male	102.14	$r = -0.767$; $P = 0.0263$; $n = 8$[a]	$r = -0.681$; $P = 0.0919$; $n = 7$
Male	93.38	$r = -0.876$; $P = 0.000877$; $n = 10$[a]	$r = -0.906$; $P = 0.00489$; $n = 7$[a]
Male	91.30	$r = -0.943$; $P = 0.00001$; $n = 11$[a]	$r = -0.941$; $P = 0.0004$; $n = 8$[a]
Male	103.42	$r = -0.927$; $P = 0.000320$; $n = 9$[a]	$r = -0.927$; $P = 0.0003$; $n = 9$[a]
Male	93.82	$r = -0.624$; $P = 0.0301$; $n = 12$[a]	$r = -0.982$; $P = 0.000002$; $n = 9$[a]
Male	104.42	$r = -0.716$; $P = 0.0132$; $n = 11$[a]	$r = -0.716$; $P = 0.0132$; $n = 11$[a]
Female	80.64	$r = -0.488$; $P = 0.267$; $n = 7$	–
Female	84.3	$r = -0.556$; $P = 0.152$; $n = 8$	$r = -0.888$; $P = 0.304$; $n = 3$
Female	94.12	$r = -0.949$; $P = 0.00388$; $n = 6$[a]	$r = -0.934$; $P = 0.0662$; $n = 4$
Female	88.39	$r = -0.901$; $P = 0.00227$; $n = 8$[a]	$r = -0.873$; $P = 0.127$; $n = 4$
Female	89.1	$r = -0.829$; $P = 0.0109$; $n = 8$[a]	$r = -0.986$; $P = 0.00206$; $n = 5$[a]
Female	86.58	$r = -0.577$; $P = 0.104$; $n = 9$	$r = 0.383$; $P = 0.524$; $n = 5$
Female	95.3	$r = -0.833$; $P = 0.0103$; $n = 8$[a]	$r = -0.832$; $P = 0.0398$; $n = 6$[a]
Female	94.38	$r = -0.723$; $P = 0.0278$; $n = 9$[a]	$r = -0.843$; $P = 0.0173$; $n = 7$[a]
Female	92.16	$r = -0.274$; $P = 0.389$; $n = 12$	$r = -0.268$; $P = 0.455$; $n = 10$

The sex, the snout-vent length and the statistical parameters of each Pearson's correlation performed between the number of ring and the ring width, considering all ring of each specimen, or only rings after sexual maturity are presented.
[a]Indicate the significant statistical result.

9 years for males, and the maximum age registered was 16 years for both sexes (Piantoni *et al.*, 2006a). The T was 1 for females and 0.77 for males and R_0 was nine offspring along the entire reproductive life of each female, considering the biannual female reproduction and the clutch size of two offspring (Ibargüengoytía, 2004).

Ten males and five females from the total sample ($n_{males} = 12$; $n_{females} = 9$) showed a negative correlation between the number of ring and the ring width (Table 2), but we found a different bone growth pattern during adulthood. After sexual maturity, males exhibited wider rings than females ($t = -2.966$, d.f. = 18, $P = 0.008$; $mean_{males} = 15.447 \pm 1.645$, $mean_{females} = 9.125 \pm 1.219$) and nine males maintained a negative correlation between the number of ring and the ring width (Table 2). On the contrary, only three females exhibited a negative correlation (Table 2). According to the reproductive stage of each female previously studied in Boretto *et al.* (2007), females seemed to present wider rings the years that were pregnant and narrower rings the years that were vitellogenic (Fig. 5a,b), although these differences were not significant (Wilcoxon, $Z = -1.400$; $P = 0.161$, $n = 8$; $mean_{vitellogenesis} = 8.317 \pm 1.195$; $mean_{gestation} = 9.169 \pm 1.784$). This tendency of alternation of wider and narrow rings was not observed in males (Fig. 5c).

Discussion

Phymaturus punae is exposed to severe physiological and environmental constraints on reproduction and growth, resulting in an adult life span of 11 years for males and 10 years for females and a low lifetime reproductive rate of 7.5 offspring per female. LRE and net reproductive rate are central to understanding the evolution of *P. punae* life histories. LRE indicates that females of *P. punae* only produce a mass of offspring approximately equal to 1.05 times her own body mass during life, significantly lower than in most lizards (mean LRE = 1.43; Charnov *et al.*, 2007).

As in other ectotherms, *P. punae* exhibits an indeterminate growth pattern, with higher growth rates in juveniles than in adults, whose growth bands become progressively thinner. Asymptotic growth models are used most often in reptiles to show the relationship between age and SVL, such as the logistic function of *P. punae*, the sigmoidal function of *P. tenebrosus* (Piantoni *et al.*, 2006a), *H. darwini* (Piantoni *et al.*, 2006b) and *Liolaemus pictus argentinus* (Gutiérrez *et al.*, 2013) from Patagonia and the von Bertalanffy function used in a small viviparous skink from Australia (James, 1991; Wapstra, Swain & O'Reilly, 2001; Dubey *et al.*, 2013). Age at maturity is a pivotal trait because it is correlated with lifetime fitness and is often more sensitive to natural selection than any other life-history trait (Stearns, 2000), as after maturity more energy is diverted away from growth, maintenance and storage and directed toward reproduction (Castanet *et al.*, 1993; Niewiaroswski, 2001; Charnov *et al.*, 2007). This reallocation of energy is represented in *P. punae* by the asymptote of the curve.

Lizards living in colder climates delay the age at maturity, investing prematuration energy into growth and maintenance,

presumably to maximize future reproduction (Tinkle *et al.*, 1970; Wapstra *et al.*, 2001; Roitberg & Smirina, 2006). *Phymaturus punae* from the highlands of the Andes have evolved delayed maturity, longer life span including a longer adult life span and a lower reproductive rate than *P. tenebrosus* from the Patagonian steppe (900 m asl; Piantoni *et al.*, 2006*a*). Females of both *Phymaturus* species mature earlier than males, benefitting females with a longer reproductive life span than males. Although, females in both species reproduce only once every 2 years, whereas males reproduce annually (Ibargüengoytía, 2004; Boretto *et al.*, 2007).

In *P. punae*, females have higher specific growth rates than males, even when adult males have longer body lengths (SVL). Instead, females of *P. punae* improve the possibilities to carry greater offspring mass and/or more by changing their body allometry after maturity toward a larger interlimb length and greater body width than males (Boretto *et al.*, 2007). It is possible that the differences in the specific growth rates between males and females of *P. punae* are adaptive to life in cold environments, where larger offspring body mass would be an advantage because of the greater challenge of surviving their first winter.

In *P. punae*, males differed remarkably from females in their investment of energy into growth and reproduction. Males invested more in growth than females but this investment decreased throughout life, as shown by the negative correlation between width of age bands (rings) and age after sexual maturity (Table 2), probably as a consequence of sexual selection for competition in male–male rivalry (Olsson *et al.*, 2002). In contrast, although bone growth in mature females was lower than in males, it did not decrease with age. In addition, bone growth seems to depend on reproductive condition in *P. punae* because a trend in an alternated faster growth occurred during pregnancy and slower growth during vitellogenesis was observed in some females (Fig. 5a,b). Such alternation in bone growth was not observed in males (Fig. 5c), probably because of their lower energetic requirements for reproduction. Spermatogenesis has a relatively low energy cost and does not occur at body temperatures below 20°C, whereas although vitellogenesis is probably less thermally constrained than spermatogenesis, it requires considerably greater amounts of energy because the caloric value of a clutch may be as much as one-half of the body of the female (Saint Girons, 1985). The bone growth alternation observed in some of the *P. punae* females suggests a bioenergetic trade-off between growth and reproduction (either during vitellogenesis or pregnancy), with vitellogenesis appearing to be more expensive than pregnancy. This is corroborated by the fact that yolk energy content represents the most significant component of reptilian reproductive effort and has a higher metabolic cost than pregnancy, at least in lecithotrophic viviparous species (Van Dyke & Beaupre, 2011).

Studies of energy allocation to reproduction suggest that longer-lived organism typically exhibit lower reproductive effort (Stearns, 2000; Charnov *et al.*, 2007) and this is in agreement with the lifetime reproductive effort in *P. punae* that was lower than in other lizards and mammals with shorter life spans as reviewed by Charnov *et al.* (2007). The lower invest-

ment in reproduction by *P. punae* was probably a consequence of the thermal restrictions of their environment, particularly, the short activity seasons available at high altitudes (Gutiérrez, Krenz & Ibargüengoytía, 2010) which restricts foraging and energy intake. In the mountains, births occur later and juveniles go into hibernation earlier than at lower altitudes (Roitberg & Smirina, 2006; Gutiérrez *et al.*, 2010). In *P. punae*, births occur at the end of the summer and hibernation begins in early autumn, although newborns show large fat bodies and intra-abdominal yolk (Boretto *et al.*, 2007). Females produce more yolk than typically is used by the developing embryo, resulting in remnant intra-abdominal yolk in the newborn that may prevent neonatal starvation under harsh environments. Remnant yolk masses probably also increases survivorship when births occur late in the activity season (Boretto *et al.*, 2007). In the present study, we point out relevant life-history traits for the lizard *P. punae*, which inhabits cold and harsh environments in the highlands of the Andes. We found that females have a relative low lifetime reproductive effort and reproductive rate. In addition, we present evidences of the sexual dimorphism in the pattern of energy allocation between growth and reproduction. Present results are relevant considering the harsh conditions of the environments that *P. punae* inhabit, the viviparous mode of reproduction of this species and the low energy herbivorous diet.

Acknowledgments

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