



Female reproductive biology of the lizards *Liolaemus sarmientoi* and *L. magellanicus* from the southern end of the world

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Lizards that live in the harsh climate of the Argentinean Patagonia (40°–53° S) are active for a period restricted to spring and summer when vitellogenesis, pregnancy and birth take place. Herein, we present data on the female reproductive cycle, body size at sexual maturity, litter size and fat-body cycle of one of the world's southernmost reptiles, *Liolaemus sarmientoi*. We also provide preliminary data on the reproductive cycle of a sympatric species, *L. magellanicus*. Females of both species start vitellogenesis in late spring, probably arrested or continued at very low rates during brumation resumed in the spring of the next year. Pregnancy starts in spring and births of *L. sarmientoi* (2 to 7 offspring) and *L. magellanicus* (3 to 4 offspring) occur over a month in midsummer. Females that give birth earlier can start a new vitellogenic cycle before autumn and perform an annual reproductive cycle. However, females which give birth later delay the start of a new vitellogenic cycle until the next spring, performing a biennial reproductive cycle. Thus, females have the potential to adjust their frequency of reproduction according to the energetic restrictions imposed by environmental conditions in the southernmost lands of South America.

Key words: annual-biennial reproductive cycle, cold climate, fat bodies, life history, *Liolaemus magellanicus*, *Liolaemus sarmientoi*, viviparity

INTRODUCTION

Reproductive cycles in reptiles are tightly dependent on temperature, photoperiod, moisture and food availability, resulting in diverse life histories (Duvall et al., 1982; Bauwens, 1999). Squamate reptiles have adapted repeatedly to different environmental conditions; viviparity has evolved independently more than 115 times (Blackburn, 2014). For example, lizards exhibit sexual or parthenogenetic reproduction (Cuellar et al., 1985) and vary the frequency of reproduction from annual to triennial reproductive cycles (Cree, 1994; Ibagüengoytía, 2008), sometimes skipping a year of reproduction (van Wyk, 1991; Ibagüengoytía & Casalins, 2007; Boretto & Ibagüengoytía, 2009). These differences in reproductive biology result in a huge variability of mean annual reproductive output (Cree, 1994) and different degrees of reproductive investment (Tinkle, 1969; Shine, 1992; Pincheira-Donoso & Tregenza, 2011).

Lizards from tropical environments usually produce one or more broods per year, while in temperate environments the reproductive cycles of lizards are characteristically seasonal, alternating periods of reproductive activity

and brumation (Duvall et al., 1982; Guillelte & Casas-Andreu, 1987). These characteristics depend not only on temperature, but also on feeding resources and the opportunity to store energy as fat which females subsequently use to perform vitellogenesis (Saint Girons, 1985; Van Wyk, 1991; Pough et al., 1998; Shine, 2003). Thus, extended cycles have been linked to the length of the activity season (Ibagüengoytía & Cussac, 1996, 1998) as well as to the energy constraints for reproduction (Van Wyk, 1991; Cree & Guillelte, 1995; Edwards et al., 2002; Hare & Cree, 2005; Boretto & Ibagüengoytía, 2006; Ibagüengoytía & Casalins, 2007). Multiannual cycles can occur as a consequence of several factors such as: prolonged vitellogenesis (Van Wyk, 1991, Cree et al., 1992, Cree & Guillelte, 1995), prolonged gestation (Vial & Stewart 1985; Cree & Guillelte, 1995; Wilson & Cree, 2003), vitellogenesis and gestation alternated between different activity seasons (Ibagüengoytía & Cussac, 1996, 1998), and/or skipping years of reproduction when females lack sufficient energy resources to start a new reproductive cycle (Van Wyk, 1991; Ibagüengoytía & Casalins, 2007; Boretto & Ibagüengoytía, 2009). Populations exhibiting these cycles are identified by the

temporal overlap of females performing vitellogenesis and females performing pregnancy (Habit & Ortiz, 1996; Ibagüengoytía & Cussac, 1996, 1998) or by the presence of a high percentage (approximately 50%) of non-reproductive adult females during the reproductive season (Aldridge 1979; Van Wyk, 1991; Ibagüengoytía & Casalins, 2007).

With at least 257 described species (Abdala & Quinteros, 2014), the genus *Liolaemus* is the most diversified genus of the family Liolaemidae. *Liolaemus* has broad latitudinal and altitudinal distributions, from the Andes of Peru to Tierra del Fuego of Argentina and Chile (from 9° to 53° S and from sea level to 5,000 m.a.s.l.); Cei, 1986; Schulte et al., 2000; Scolaro, 2005). Members of this genus show a high diversity of physiological responses to adapt to a high diversity of environments and climates (Ibagüengoytía et al., 2008; Cruz et al., 2009; Medina et al., 2012; Corbalán et al., 2013; Moreno-Azócar et al., 2013).

Patagonian Liolaemids (between 40° and 53° S) have followed distinct evolutionary paths in response to the cold-temperate climate, with marked seasonality in temperature; they are typically active in spring and summer months but live in brumation for approximately 7 months from early autumn to mid-spring (Ibagüengoytía, 2008). In addition, their life histories can differ greatly depending on environmental conditions and reproductive mode (oviparous or viviparous), diet (herbivorous, insectivorous or omnivorous), and habit (saxicolous, psammophilous or arboreous) (Cei, 1986; Scolaro, 2005). Most *Liolaemus* species have annual female reproductive cycles (see review in Ibagüengoytía, 2008; Medina & Ibagüengoytía, 2010), with the exception of the facultative annual to biennial cycle of *L. elongatus*, which inhabits rocky outcrops in the steppe and in the forest-steppe transition environments from 39° to 41.5° S (Ibagüengoytía & Cussac, 1996, 1998) and the biennial to triennial cycle of *L. pictus*, which inhabits the Andean-Patagonian forest between 529 and 1600 m a.s.l.

In this study we present the female reproductive cycle of the two southernmost species of lizards. *Liolaemus sarmientoi* is medium sized (mean snout-vent length, SVL=77 mm; Ibagüengoytía et al., 2010), and is

distributed in the central and southern portions of Santa Cruz province (Argentina) from 48° S, 70° W to 52° S, 69° W (Breitman et al., 2014). It is omnivorous, saxicolous, and births were reported to occur in January and February with a litter size between 3 and 5 neonates (Cei, 1986; Scolaro & Cei, 1997; Scolaro, 2005). *Liolaemus magellanicus* is a small lizard (mean SVL=56 mm; Ibagüengoytía et al., 2010) distributed in small populations from southern Santa Cruz province (50° S, 71° W; Argentina) to the northern portion of Tierra del Fuego island (53° S, 68° W; Argentina and Chile), where it is the only species of reptile observed (Donoso-Barros, 1966; Jaksic & Schwenk, 1983; Scolaro, 2005; Breitman et al., 2014). This species is insectivorous and psammophilous (Cei, 1986; Scolaro, 2005). A previous study of *L. magellanicus* by Jaksic & Schwenk (1983) reported that births occur during midsummer (January or February). The authors argued that *L. magellanicus* might therefore have a gestation period of 11 or 12 months, or that sperm storage occurs. In addition, *L. sarmientoi* and *L. magellanicus* show the lowest mean field body temperature in liolaemids (26.18°C and 23.35°C, respectively, Ibagüengoytía et al., 2010), well below their optimal locomotor performance temperatures (Fernández et al., 2011). Herein, we study the female reproductive biology of *L. sarmientoi* and show preliminary results for *L. magellanicus*. In particular, size at sexual maturity, timing of vitellogenesis, pregnancy and birth, litter size, offspring size, egg size in-utero and the cycle of fat deposition are discussed in relation to environmental restraints on the female reproductive cycle.

MATERIALS AND METHODS

Study area, climate and specimens

Field work was carried out in Santa Cruz province, Argentina (50° S, 72° W and 51° S, 70° W; 133 m a.s.l.), where the climate is cold temperate and semiarid (Soto & Vázquez, 2001). Precipitation is regularly distributed throughout the year, ranging between 200 and 400 mm per year, with a moderate Atlantic influence from the coast (Soto & Vázquez, 2001). The mean annual air temperature is 8.04°C (ranging from 1.2 to 14.1°C), and

Table 1. Follicular size and snout-vent length (SVL, mm) of juvenile, vitellogenic, postpartum and pregnant *Liolaemus sarmientoi* and *L. magellanicus* females. Mean, standard error (\pm SE), range, and sample size (n) are indicated.

	Juvenile female		Vitellogenic female		Postpartum female		Pregnant female	
	Mean (\pm SE)	Range	Mean (\pm SE)	Range	Mean (\pm SE)	Range	Mean (\pm SE)	Range
<i>Liolaemus sarmientoi</i>								
Follicular size	1.22 (\pm 0.04)	1.16–1.28	3.04 (\pm 0.20)	1.53–5.98	2.62 (\pm 0.10)	1.89–3.54		
SVL	55.38 (\pm 0.36)	54.68–55.88	73.58 (\pm 1.76)	57.22–97.68	79.45 (\pm 0.91)	71.80–87.03	76.33 (\pm 0.67)	65.08–89.13
n	3		26		20		57	
<i>Liolaemus magellanicus</i>								
Follicular size	0.84 (\pm 0.09)	0.66–0.96	2.8 (\pm 0.46)	0.92–5.37	2.18 (\pm 0.19)	1.77–2.59		
SVL	37.41 (\pm 0.59)	36.6–38.56	53.35 (\pm 1.69)	43.52–61.58	55.15 (\pm 4.14)	48.08–66.98	55.73 (\pm 2.16)	52.01–59.50
n	3		12		4		3	

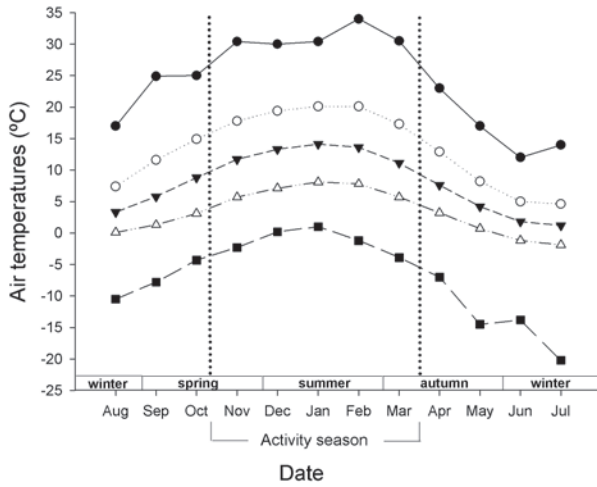


Fig. 1. Air temperatures according to the Meteorological Station of Río Gallegos Airport, Santa Cruz (51° 37' S, 69° 17' W and 19 m a.s.l.) corresponding to the period 1981–1990: maximum and minimum extremes (● and ■), maximum and minimum means (○ and △), and mean (▼) air temperatures throughout the year. Vertical dotted lines indicate lizards' activity season. Seasons of the southern hemisphere (spring, summer, autumn and winter) are also indicated. Adapted from Fernández & Ibarquengoytia (2012).

the mean air temperature during the lizards' activity period from October to March is 12.1°C (Meteorological Station of Río Gallegos, Santa Cruz; Fig. 1). Seasonal references (spring, summer, autumn and winter) refer to seasons of the southern hemisphere.

Females of *L. sarmientoi* ($n=54$) and *L. magellanicus* ($n=13$) were caught by hand or noose from November to February in 2007, 2009 and 2011. Lizards were brought to the laboratory where we measured body mass (Ohaus, Scot Pro ± 0.01 g) and snout-vent length (SVL; with a Vernier caliper ± 0.01 mm). The specimens were euthanised by intra-peritoneal administration of sodium thiopental, fixed in Bouin solution for 24 hours and preserved in 70% ethanol. The specimens are stored in the herpetological collection of Department of Zoology at Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, San Carlos de Bariloche, Argentina (CRUB-UNC). Capture permission was obtained from the Wildlife Delegation of Santa Cruz province, Argentina, according to permit number 09/09. Lizards were cared following the ASIH/HL/SSAR Guidelines for Use of Live Amphibians and Reptiles, as well as the regulations detailed in the Argentinean National Law n° 14346.

In addition we also included all available museum specimens from Argentina of *L. sarmientoi* ($n=12$) and *L. magellanicus* ($n=10$) deposited in the LJAMM-CNP herpetological collection, Centro Nacional Patagónico (CENPAT), Puerto Madryn, Argentina and from the herpetological collection of Instituto de Herpetología, Fundación Miguel Lillo, Tucumán, Argentina. The specimens were collected in the same area as our captures from December to February 2003, 2007 and 2009. One lizard was captured in October 2003 (a juvenile *L. magellanicus*). Therefore the total number

of specimens used for the analyses was $n=66$ for *L. sarmientoi* and $n=23$ for *L. magellanicus*.

For the estimation of body sizes of pregnant females and for the estimation of litter and offspring size, we used pregnant females of *L. sarmientoi* ($n=42$) and *L. magellanicus* ($n=3$), captured in December 2009 and January 2014. These females gave birth in the laboratory during captivity and subsequently were released at their respective capture sites. These 45 females were not used to determine the pregnancy duration and time of births because captivity can modify the timing of the reproductive events. Due to the low sample size some statistical analyses were not performed for *L. magellanicus*.

Female reproductive cycle, sexual maturity and fat-body cycle

Reproductive condition was determined by examination of the macroscopic morphology of ovarian follicles and uteri. Follicular size, estimated as the diameter of the largest follicle, was measured with a Vernier caliper (± 0.01 mm) using an Olympus SZ 40 stereo microscope.

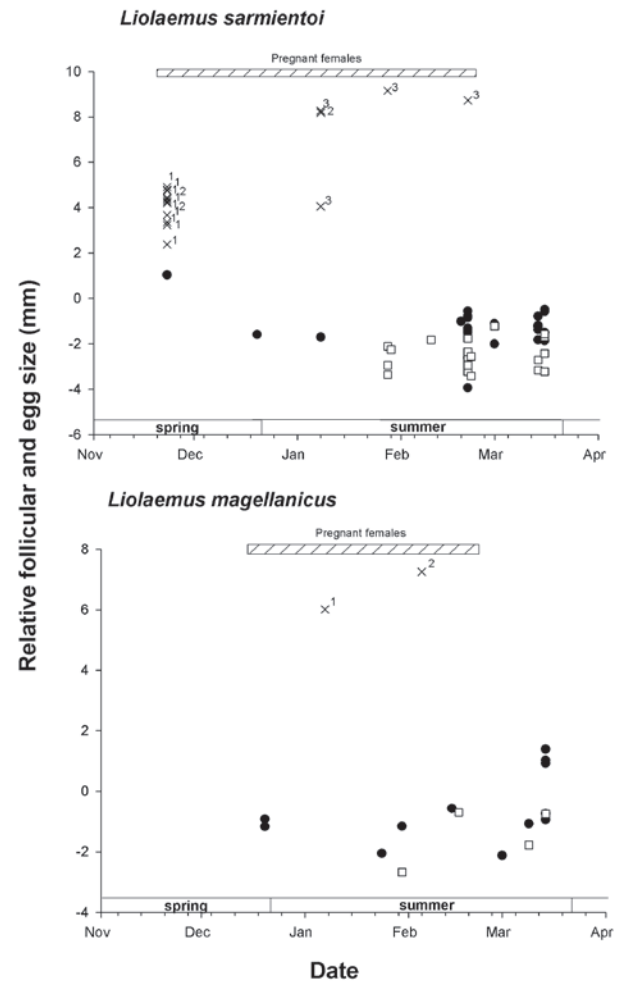


Fig. 2. Relative follicular and egg size of *Liolaemus sarmientoi* and *L. magellanicus* during the activity period: vitellogenic females (●), postpartum females (□), and eggs (x). Numbers indicate the embryonic development stage (1 early, 2 medium, and 3 advanced) and horizontal bar represents the period when pregnant females were observed.

The relative follicular size was calculated as the residuals of the linear regression of follicular size on SVL. Pregnant female follicles were not measured to preserve the tissues for further placentation studies. By examination of the macroscopic morphology, uteri were categorised into Type 1 (thread-like oviduct, transparent and without folds, typical of juveniles *sensu* Van Wyk, 1991), Type 2 (distended with medium-size folds over the entire surface, indicative of adulthood and vitellogenic recrudescence *sensu* Vitt & Caldwell, 1993), or type 3 (more stretched than Type 2, with numerous large folds over the entire surface and/or presence of corpora lutea, indicative of recent parturition *sensu* Flemming & Van Wyk, 1992; Ramirez-Pinilla, 1992).

Minimum SVL at sexual maturity in females was estimated from the smallest female containing signs of reproductive activity such as vitellogenic follicles, embryos in uteri or Type 2 and 3 uteri (Boretto & Ibarguengoytía, 2006). Based on the integration of gonads morphology and reproductive tract (uteri), we classified females (*sensu* Medina & Ibarguengoytía, 2010) as juvenile (uteri Type 1 and pre-vitellogenic follicles smaller than 1.5 mm in *L. sarmientoi* and smaller than 1 mm in *L. magellanicus*); vitellogenic (uteri Type 2

and yellow follicles larger than 1.5 mm in *L. sarmientoi* and larger than 1 mm in *L. magellanicus*); or postpartum (uteri Type 3, follicles smaller than 4 mm in *L. sarmientoi* and smaller than 3 mm in *L. magellanicus*, with corpora lutea present).

Fat bodies were excised from adult females, weighed (Ohaus balance, Scout Pro ± 0.01 g), and fixed in 10% formaldehyde. Relative fat-body mass were calculated as the residuals of the regression of fat-body mass on SVL.

Egg size in uteri, stages of embryonic development, litter size, offspring size, frequency of reproduction and mean annual reproductive output

We use the term "egg" to refer to eggs (with embryos) in the uterus of a viviparous species. Egg size was estimated as the longest diameter of the largest egg (measured with a Vernier caliper, ± 0.01 mm) because all eggs kept the same ovoid form throughout the development. Three stages of embryonic development were recognised (*sensu* Leyton et al., 1980): early (1, from cleavage to neurula or embryos with somites), medium (2, from curved column to embryos with developed limbs, and the presence of abundant yolk), and advanced (3, foetuses with scales and pigmentation). Litter size was considered as the mean of the total number of embryos in uteri obtained from autopsied pregnant females and from females that gave birth in laboratory. Offspring size was considered as mean SVL (measured with a Vernier caliper ± 0.01 mm).

The frequency of reproduction was calculated as the number of litters produced per season. We define an annual reproductive cycle as one in which reproduction occurs every year and a biennial reproductive cycle as one producing one litter every two years (Ibarguengoytía, 2008). The mean annual reproductive output was calculated as the product of the mean litter frequency (mean number of litters per year per mature sized female) and the mean annual litter size (Cree, 1994).

Statistical analyses

We used the statistical software Sigma Stat v.3.5[®], SPSS v.15.0[®] and Sigma Plot v.10.0[®] for statistical analyses. Standardised residuals of the regressions between follicular size on SVL were calculated to remove the effect of body size from the analyses. For the purpose of comparison with relative follicle sizes, we calculated the residuals of the regression of fat-body mass on SVL.

Dependence between variables was analysed by simple Linear Regressions. Dates of capture were considered as days of a single standard year (Ibarguengoytía & Cussac, 1999). Differences between means of two samples were analysed using *t*-tests, for more than two tests we used an Analysis of Variance (One-Way ANOVA) with a Holm-Sidak method as an a posteriori test, and ANCOVA when including a covariable in the model. Assumptions of normality and homogeneity of variance were checked using Kolmogorov-Smirnov and Levene's tests, respectively.

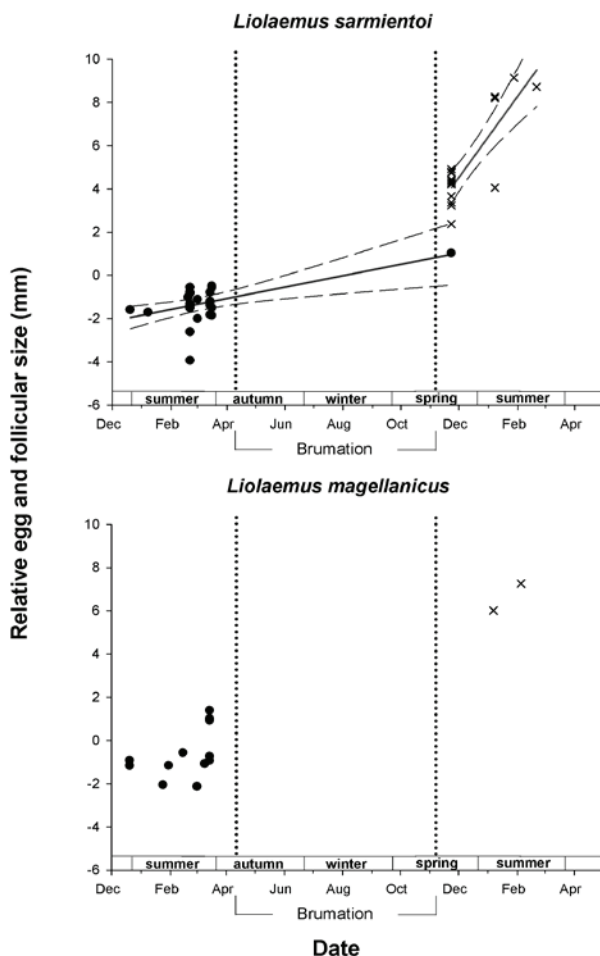


Fig. 3. Linear regression (solid line) and 95% confidence intervals (dashed lines) of relative vitellogenic follicular size (●) and egg size (x) of *Liolaemus sarmientoi* and *L. magellanicus* throughout the year. Vertical dotted lines indicate brumation period.

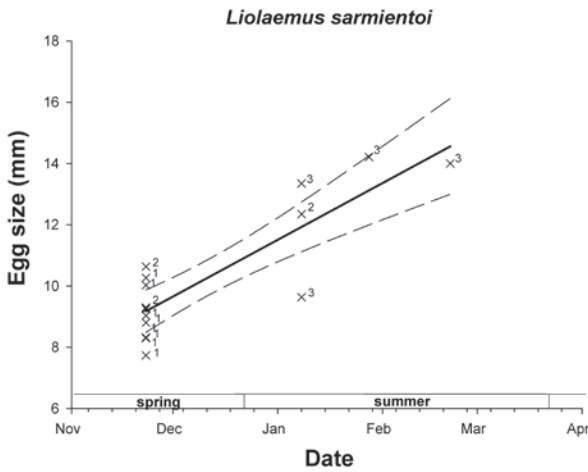


Fig. 4. Relationship between *Liolaemus sarmientoi* egg size (x) and date. Early (1), medium (2) and advanced (3) stage of embryonic development, linear regression (black solid line) and 95% confidence intervals (dashed lines) are indicated.

RESULTS

Female size at sexual maturity

In *L. sarmientoi* and *L. magellanicus*, size at sexual maturity of Type 2 females was 57.22 mm and 43.52 mm SVL, respectively (Table 1). The mean size of adult (vitellogenic, pregnant and postpartum females) *L. sarmientoi* was 76.24 mm SVL (± 0.63 , $n=103$, range 57.22–97.68), and the mean size of adult *L. magellanicus* was 54.11 mm SVL (± 1.36 , $n=19$, range 43.52–66.98). Follicular size and SVL of juveniles, vitellogenic, pregnant and postpartum females of both species are shown in Table 1.

Female reproductive cycle

In both species, follicular size of vitellogenic females were related to SVL (Linear Regressions: $F_{L. sarmientoi 1, 25} = 24.30$, $r^2=0.50$, $p<0.001$; $F_{L. magellanicus 1, 11} = 11.52$, $r^2=0.53$, $p=0.007$). In postpartum females of *L. sarmientoi* follicular size was not related to SVL ($F_{1, 19} = 0.43$, $r^2=0.02$; $p=0.521$).

Liolaemus sarmientoi pregnant females ($n=59$) were found from from late spring (late November) to late summer (late February, Fig.2). Pregnant *L. magellanicus* females ($n=7$) were found from mid-December to late summer (late February, Fig. 2). In both species, births started in midsummer (late January), coinciding with the first observation of postpartum females (Type 3; Fig. 2) and lasting until late February. Parturition by pregnant captive females ($n=45$) was not considered because captivity can influence the timing of the reproductive events. The follicles observed in pregnant females were smaller than the smallest follicle measured in vitellogenic females of *L. sarmientoi* and *L. magellanicus* (1.53 mm and 0.92 mm, respectively).

Liolaemus sarmientoi postpartum females did not show a noticeable increment in vitellogenic follicular size with date of capture after parturition (linear regression: $F_{1, 19} = 0.27$, $r^2=0.01$, $p=0.612$). The date of parturition was not related to differences in female SVL of postpartum females of *L. sarmientoi* captured in January, February,

or March (one-way ANOVA: $F_{2, 19} = 0.25$, $p=0.781$; Mean $SVL_{January} = 78.16 \pm 2.69$ mm, $n=4$; Mean $SVL_{February} = 79.92 \pm 4.28$ mm, $n=10$; and Mean $SVL_{March} = 79.53 \pm 4.83$ mm, $n=6$).

Reproductive females of *L. sarmientoi* showed an increase in follicular size due to vitellogenesis from the end of spring (December) until late spring of the next year (late November) after the brumation period ($F_{1, 25} = 11.00$, $r^2=0.31$, $p=0.003$; Fig. 3). There was an increase in egg size, according to different stages of development, from late spring to late summer ($F_{1, 15} = 35.11$, $r^2=0.72$, $p<0.001$; Fig. 3, 4). These analyses were not performed for *L. magellanicus* due to the low sample size (but see Fig. 3).

Egg size in uteri, stages of embryonic development, litter size, offspring size, frequency of reproduction and mean annual reproductive output

Liolaemus sarmientoi had a mean egg size of 10.28 ± 0.52 mm ($n=16$, range 7.74–14.21 mm). The stages of embryonic development observed throughout the pregnancy period were early ($n=9$), medium ($n=3$) and advanced ($n=4$, Fig. 4). In *L. magellanicus*, two females were pregnant, and had mean egg sizes of 9.24 and 10.99 mm with an early and medium embryonic developmental stage, respectively. Egg size *L. sarmientoi* differed between early and advanced stages of embryonic development ($F_{2, 15} = 11.01$, $p=0.002$; Mean $_{early} = 9.00 \pm 0.82$ mm, $n=9$; Mean $_{medium} = 10.75 \pm 1.55$ mm, $n=3$; and Mean $_{advanced} = 12.80 \pm 2.14$ mm, $n=4$; Holm-Sidak method: $t_{early vs. advance} = 4.64$, $p<0.001$, $t_{advance vs. medium} = 1.98$, $p>0.05$, $t_{medium vs. early} = 1.92$, $p>0.05$). Pregnant females of *L. sarmientoi* did not show a relationship between SVL and egg size ($F_{1, 15} = 0.07$, $r^2=0.005$, $p=0.798$) and between SVL and offspring SVL ($F_{1, 13} = 4.50$, $r^2=0.273$, $p=0.055$).

Liolaemus sarmientoi had a mean litter size of 4.12 ± 0.18 (pregnant females $n=57$, litter size range 2–7 embryos), and *L. magellanicus* had a mean litter size of 3.40 ± 0.24 (pregnant females $n=5$, litter size range 3–4 embryos). *Liolaemus sarmientoi* litter sizes were related to female SVL ($F_{1, 56} = 14.77$, $r^2=0.21$, $p<0.001$). Mean offspring SVL

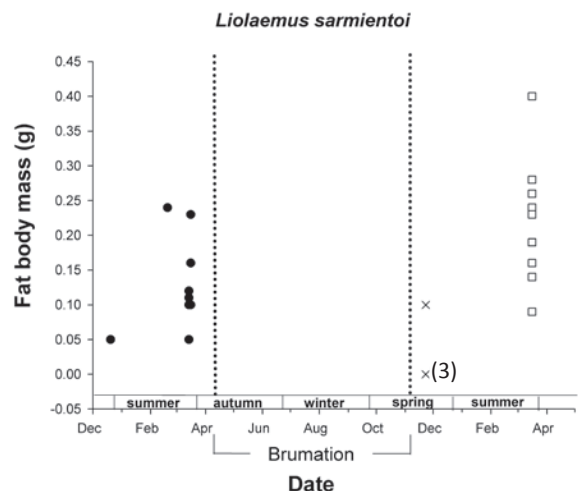


Fig. 5. Fat-body masses of vitellogenic (●), pregnant (x) and postpartum females (□) of *Liolaemus sarmientoi* throughout the year. Vertical dotted lines indicate brumation period of patagonian lizards. Number in parentheses denotes sample size.

($n_{\text{pregnant females}}=14$) was 31.52 ± 0.32 mm and mean offspring total length (SVL + Tail length) was 67.14 ± 0.86 mm. Reproduction of *L. sarmientoi* was annual to biennial and its mean annual reproductive output was 3.09 offspring/female/year. In *L. magellanicus*, reproduction was annual to biennial and its mean annual reproductive output was 2.55 offspring/female/year.

Fat-body cycle

In vitellogenic and postpartum females of *L. sarmientoi*, fat body mass was not related to SVL ($F_{1,18}=4.20$, $r^2=0.198$, $p=0.056$). *Liolaemus sarmientoi* showed larger fat body masses in vitellogenic females during spring-summer. The smallest fat body masses were found in pregnant females, increasing after parturition the next summer (Fig. 5). Fat body masses differed between vitellogenic, pregnant and postpartum females of *L. sarmientoi* ($F_{2,22}=9.86$, $p=0.001$; Mean_{vitellogenic} = 0.12 ± 0.07 g, $n=10$; Mean_{postpartum} = 0.22 ± 0.09 g, $n=9$; and Mean_{pregnant} = 0.02 ± 0.05 g, $n=4$; Holm-Sidak method: $t_{\text{postpartum vs. pregnant}}=4.26$, $t_{\text{postpartum vs. vitellogenic}}=2.85$, $t_{\text{vitellogenic vs. pregnant}}=2.12$, $p<0.05$). In *L. magellanicus* such changes were not noticeable ($n_{\text{vitellogenic}}=1$, $n_{\text{postpartum}}=4$). In both species, pregnant females showed small (≤ 0.1 g) or no fat bodies.

There was no relationship between relative follicle size and relative fat body mass in vitellogenic females of *L. sarmientoi* ($F_{1,9}=4.08$, $r^2=0.34$, $p=0.078$) or in postpartum females of *L. sarmientoi* ($F_{1,8}=1.30$, $r^2=0.16$, $p=0.291$). Fat-body masses of *L. sarmientoi* were not different between vitellogenic and postpartum females (ANCOVA: $F_{2,18}=3.51$, $p=0.054$; Mean_{vitellogenic} = 0.12 ± 0.02 g, $n=10$; Mean_{postpartum} = 0.22 ± 0.03 g, $n=9$), using SVL as a covariable in the analysis ($t\text{-test}_{\text{vitellogenic vs. postpartum}}: t_{17}=-4.62$, $p<0.001$).

DISCUSSION

Liolaemus sarmientoi females reproduce annually or biennially, with a 9–10 month duration of vitellogenesis (from late spring to ovulation during the following spring after the brumation period). Despite a low sample size our data suggest a similar annual-biennial cycle for *L. magellanicus*. The presence of postpartum females with significant variation in follicular size among females in late January shows that some lizards give birth early in the activity season, being able to start a new vitellogenic cycle before brumation, performing an annual reproductive cycle. This reproductive pattern characterised by vitellogenesis, pregnancy and birth in spring and summer has been observed in most oviparous and viviparous *Liolaemus* in harsh cold environments (see review in Ibagüengoytia, 2008; Medina & Ibagüengoytia, 2010).

Some pregnant females of *L. sarmientoi* and *L. magellanicus* give birth at the end of the activity season (late February) and delay vitellogenesis until the next year, indicating that these females perform a biennial cycle. Biennial reproductive cycles are characterised by females spending an entire season conducting vitellogenesis, and then becoming pregnant in the spring of the next year. We assume that vitellogenesis does not occur simultaneously with pregnancy because neither *L. sarmientoi* nor *L. magellanicus* pregnant females

were found with enlarged vitellogenic follicles. These results are concordant with the inhibition of hepatic vitellogenin synthesis by progesterone as reported in *Liolaemus* and other reptiles (Callard et al., 1992; Leyton & Valencia, 1992). We postulate a facultative biennial cycle for *L. sarmientoi*, based on the simultaneous presence of early-pregnancy females and vitellogenic females. Moreover, the facultative option is not limited by female body size in either species: larger as well as smaller adult females can perform an annual or a biennial cycle. This type of facultative life history has been reported only in the Patagonian species *L. elongatus* (Ibagüengoytia & Cussac, 1998), *Phymaturus zapalensis* (Boretto & Ibagüengoytia, 2009) from northern Patagonia (41° – 39° S, respectively) and in the gecko *Homonota darwini* (32° – 52° S, Ibagüengoytia & Casalins, 2007).

The length of the brumation period enables to complete vitellogenesis and ovulation in several reptiles living in cold climates (Duvall et al., 1982; Gavaud, 1983; Whittier & Tokarz, 1992). Those *L. sarmientoi* females who ovulate and mate after brumation show an embryonic development period of four months, while the gestation period of *L. magellanicus* lasts a minimum of three months (from December to late January or late February). Births of *L. sarmientoi* and *L. magellanicus* start in mid-summer (Ceï, 1971; Jaksic & Schwenk, 1983). The absence of pregnant females shortly before and after brumation and the absence of newborns in early spring suggests that there is no over-winter pregnancy in *L. sarmientoi* (consistent with observations on congeneric species, Ibagüengoytia, 2008), corroborating the hypothesis that sperm is stored during winter (Jaksic & Schwenk, 1983).

Regardless of female size, egg size in *L. sarmientoi* increases with embryonic development throughout the activity period. This may be due to liquid absorption (Lemus et al., 1981) or could be the result of a partial placentotrophy consisting of additional water and inorganic nutrient exchange between mother and embryo (as observed in *L. elongatus*, Crocco et al., 2008). Moreover, litter size of *L. sarmientoi* is related to female size. The litter size documented for *L. magellanicus* is lower than previously reported (Jaksic & Schwenk, 1983; Ceï, 1986), which could be linked to smaller females SVL in the present study. Fat body cycles of *L. sarmientoi* females are closely coupled with the reproductive cycle. Pregnant females have few if any fat bodies, suggesting that stored lipids are necessary to perform vitellogenesis.

Reproductive success requires the coordination of internal processes with external events (Saint Girons, 1985). Thus, the reproductive biology of *Liolaemus* species has evolved to respond to conditions imposed by a cold-temperate, harsh and fast-changing weather of the Southern Cone of South America. *Liolaemus sarmientoi* and *L. magellanicus* females have evolved plastic life histories, involving the ability to adjust the reproductive timing to climate and energetic resources. The either annual or biennial cycle enable birth in early summer to maximise the potential of offspring to grow before their first winter, and to enable females to increase their lipid reserves during summer to start a new vitellogenic cycle.

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