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Viviparity Advantages in the Lizard *Liolaemus sarmientoi* from the End of the World

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Abstract Two hypotheses have prevailed to explain the evolution of viviparity in reptiles: the first proposed that viviparity evolved in response to cold-climates because the possibility of pregnant females to thermoregulate at higher temperatures than embryos could experience in a nest in nature. The second hypothesis posits that the advantage of viviparity is based on the possibility of females to maintain stable body temperatures during development, enhancing offspring fitness. With the aim to contribute to understanding the origins of viviparity in reptiles, we experimentally subjected pregnant females of the austral lizard *Liolaemus sarmientoi* to two temperature treatments until parturition: one that simulated environmental temperatures for a potential nest (17–25 °C) and another that allowed females to thermoregulate at their preferred body temperature (17–45 °C). Then, we analysed newborn body conditions and their locomotor performance to estimate their fitness. In addition, we measured the body temperature in the field and the preferred temperature in the laboratory of pregnant and non-pregnant females. Pregnant females thermoregulated to achieve higher temperatures than the environmental temperatures, and also thermoregulated within a narrower range than non-pregnant females. This could have allowed embryos to develop in higher and more stable temperatures than they would experience in a nest in nature. Thus, offspring developed at the female preferred temperature showed greater fitness and were born earlier in the season than those developed at lower environmental temperatures.

Herein, we show that results are in agreement with the two hypotheses of the origin of viviparity for one of the southernmost lizards of the world.

Keywords Evolution of viviparity · Locomotor performance · Pregnant females · Thermoregulation · Viviparity hypotheses

Introduction

In Squamate reptiles viviparity evolved from oviparity in at least 115 different lineages (Blackburn 2015), and is thought to have occurred through a gradual increase of egg retention in uterus, the calcareous eggshell reduction into a fibrous membrane, and the origin of placentas, culminating with the birth of fully developed neonates (Packard et al. 1977; Shine and Bull 1979; Qualls and Shine 1998; Stewart and Blackburn 2014). In this way, and because they have a great phenotypic plasticity, Squamate reptiles are an appealing model to study the selective forces of the transition from oviparity to viviparity and to know its adaptive significance (Elphick and Shine 1998; Blackburn 2000; Lambert and Wiens 2013; Stewart and Blackburn 2014). The hypotheses of the evolution of viviparity in reptiles agree with the idea that viviparity in Squamata has evolved for thermal reasons. These hypotheses postulate that in climates with extreme temperatures the thermal difference between uterus and nest, produced by maternal thermoregulation, provides an advantage and is a key factor in the evolution of viviparity (Blackburn 2000). Thus, the *cold-climate hypothesis* proposes that viviparity has evolved in response to cold-climates due to the possibility of pregnant females, by means of thermoregulation, to keep higher temperatures in uterus than embryos could experience in

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a nest in nature (Mell 1929; Blackburn 1982; Shine 1983, 1985). For many years, this hypothesis was supported by the observation that viviparity in reptiles is relatively common in cold-climates (Shine 1985; Qualls and Shine 1998; Lambert and Wiens 2013); however, this hypothesis does not explain why viviparity is also a successful strategy in tropical climates, and does not take into account the effect of the thermal variability in the embryonic development. Therefore, the *maternal manipulation hypothesis* is complementary and suggests that the viviparity advantage lies in the possibility of pregnant females, to maintain more stable temperatures and in a more suitable range than those available in a nest located in the environment in which they live. This enhance the newborn fitness (Shine 1995, 2004), being a possible advantage in both cold-climates and in the tropics with extreme high temperatures (Webb et al. 2006).

In order to infer the advantages of viviparity over oviparity in reptiles becomes crucial to know how temperature influences the physiological performance of individuals, particularly neonates (Huey et al. 2009). Numerous studies show that thermal conditions experienced by reptile embryos during development have significant implications for growth, weight, body size, behaviour, thermoregulatory preferences, and running speed, ultimately affecting the offspring survival and fitness (Elphick and Shine 1998; Shine and Downes 1999; Wapstra 2000; Du and Ji 2003; Shine 2004; Ji et al. 2007a; Li et al. 2009; Gao et al. 2010; Yan et al. 2011; Lorigoux et al. 2012; Dayananda et al. 2016). Embryos developed in laboratory at very low and constant experimental temperatures, generally exhibit low hatching success, or were born late in the activity season (Rock and Cree 2003; Deeming 2004; Lourdais et al. 2004), with low body conditions (Qualls and Andrews 1999; Booth et al. 2000), and low locomotor performance (Du and Ji 2003; Gao et al. 2010; Wang et al. 2014). However embryos developed at too high and constant temperatures are generally unviable or malformed (Du and Ji 2003; Rock and Cree 2003; Deeming 2004; Gao et al. 2010; Rodríguez-Díaz et al. 2010; Wang et al. 2014). Therefore, in various studies of lizards (Braña and Ji 2007; Lin et al. 2008; Li et al. 2009; Rodríguez-Díaz et al. 2010; Yan et al. 2011; Paranjpe et al. 2013; Cadby et al. 2014; Woolrich-Piña et al. 2015) and snakes (Charland and Gregory 1990; Tu and Hutchison 1994; Charland 1995; Chiaraviglio 2006; Webb et al. 2006; Gao et al. 2010) the difference in the thermoregulatory behaviour of pregnant or gravid females vs. non-pregnant or non-gravid females is attributed to a strong commitment of pregnant females to thermoregulate more precisely, between an optimal range of body temperatures, for the proper development of embryos. In this sense, viviparous females, unlike oviparous ones, have more control over the development and fitness of their offspring. This is because viviparous females are able to thermoregulate to maintain

their body temperature in an optimal range during the entered development of embryos (Wapstra et al. 2010; Wang et al. 2014), and precisely modify the timing of reproduction according to the climate they inhabit (Crews and Gans 1992; Atkins et al. 2007).

The lizards of genus *Liolaemus* has a broad latitudinal and altitudinal distribution, from the Andes of Peru to Tierra del Fuego in Argentina and Chile (from 10°S to 54°30'S, and from sea level to more than 5000 m above sea level, m a.s.l.; Bottari 1975; Schulte et al. 2000; Aparicio and Ocampo 2010; Aguilar et al. 2013). *Liolaemus* shows high versatility in physiological responses to adjust to the broad range of environments and climates they inhabit (Cruz et al. 2009; Medina et al. 2012; Moreno-Azócar et al. 2013). In this study, with the aim to contribute to understanding the origins of viviparity in reptiles, we experimentally assessed the influence of environmental temperatures on reproductive success and offspring phenotype in the viviparous lizard *Liolaemus sarmientoi* Donoso-Barros (1973), one of the southernmost species of lizard of the world, restricted geographically to the extreme south of the cold Patagonian Magellan steppe. Thereby, to discuss the application of two hypotheses that explain the evolution of viviparity in reptiles, we compared in this species the effects of two different temperature treatments on pregnant females and their newborns: one that simulated environmental temperatures for a potential nest of an oviparous *Liolaemus* species, and another that allowed pregnant females to thermoregulate at their preferred body temperature. In addition, we compared the field activity temperature, the preferred body temperature and the effectiveness of thermoregulation between pregnant and non-pregnant adult females.

Materials and Methods

Species

Liolaemus sarmientoi is a medium sized lizard species (mean snout-vent length, SVL_{females}: 76.90 ± 1.21 mm; SVL_{males}: 76.82 ± 2.02 mm; Ibargüengoytía et al. 2010), distributed between 48°S, 70°W and 52°S, 69°W (Breitman et al. 2014) from the central portions of Santa Cruz Province, Argentina, reaching the Strait of Magellan to the South. It is omnivorous, saxicolous, viviparous, and gives birth from late January to February from 2 to 7 neonates (Cei 1986; Scolaro and Cei 1997; Fernández et al. 2015). Females of *L. sarmientoi* show plasticity in their reproductive cycles which can be either annual or biennial according to environmental conditions (Fernández et al. 2015). Regarding thermal physiology *L. sarmientoi* lizards prefer, as other *Liolaemus*, high body temperatures in laboratory

($34.4 \pm 0.28^\circ\text{C}$) and in nature they behave as poor thermoregulators (*sensu* Hertz et al. 1993; $E=0.30$) reaching a mean body temperature of 26°C , even when they experience a mean air temperature of 12°C during spring and summer (Ibargüengoytía et al. 2010). This difference between preferred body temperature and those they are able to achieve in nature is probably compensated by the possibility of reaching a maximum locomotor performance in a wide range of body temperatures (Fernández et al. 2011).

Captured Specimens

Pregnant females of *L. sarmientoi* ($N=30$) were caught from January 9th to 12th 2014 by hand or noose in the south of Santa Cruz Province, Argentina (51°S , 69°O ; 109 m a.s.l.), latitude where oviparous reptiles are not present (Breitman et al. 2014). Every capture micro-site was geo-referenced (GPS data 3 m resolution, GARMIN) in order to release lizards in the exact place where they were captured. Sex was determined by the absence of pre-cloacal glands, and the reproductive status (pregnant or non-pregnant) was determined by palpation. Captures were made when females were estimated to be from 4 to 6 weeks of pregnancy (medium stage of embryonic development) based on the knowledge of the *L. sarmientoi* female reproductive cycle (Fernández et al. 2015). Research permission (Permit No 013/2012) was obtained from the Wildlife Delegation of Santa Cruz Province, Argentina. 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.

Recorded Field Data

During captures body temperatures were recorded (T_b ; TES 1303, $\pm 0.03^\circ\text{C}$ thermometer) using a thermocouple (TES TP-K01, 1.62 mm of diameter) inserted ± 1 cm inside the cloaca. All individuals were captured in activity, outside their shelters, carrying out any activity related to foraging, social behaviour or thermoregulation. The micro-environmental temperatures (TES 1303, $\pm 0.03^\circ\text{C}$ thermometer) were also recorded at the capture sites in order to determine the thermal resources used by lizards for thermoregulation by measuring substratum temperature (T_s) and air temperature at one centimetre above the ground (T_a).

Operative Temperatures (T_e)

T_e for Lizards Thermoregulation

The operative temperatures (T_e) represents the "null" distribution of T_b than non-regulating animals would achieve in their environment (Hertz et al. 1993). T_e was measured in a

variety of micro-sites in which *L. sarmientoi* inhabit, using grey PVC models (1.5×8 cm) to mimic an *L. sarmientoi* adult. Thus, we estimated the temperature that an individual would experience in its environment (like under the sun, the shadow, rock or inside a shelter). The model was chosen to represent a living animal and was validated for *Liolaemus* species of similar shape and size to *L. sarmientoi*, showing a strong association between the model temperature and the live animal temperature ($r^2 > 0.91$; Kubisch et al. 2016). Each model was connected to a thermistor and sealed at the end with silicone (Fastix®). In order to record operative temperatures, six thermal models were connected to three data loggers (HOBO Onset Computer Corporation) which recorded the temperature every 30 s during field work. In addition, two thermal models connected to a data logger (HOBO Onset Computer Corporation) were set from early January to mid-March recording the micro-environmental temperature every 5 min during day and night.

T_e for Potential Nests

In order to find the temperature an oviparous *Liolaemus* egg would experience in a potential nest located in the environment where *L. sarmientoi* inhabits, seven thermistors connected to four data loggers (HOBO Onset Computer Corporation) were placed under shelters and also buried from 10 to 15 cm deep under rocks or bushes, as potential sites used by lizards to oviposition. The data loggers were set from early January to mid-March and they recorded temperature every 5 min during day and night. The mean temperature obtained from each thermistor was used to obtain the operative temperature (T_e) that an egg would experience in this environment at each potential nest.

Pregnant Females Experiments

Lizards were brought to the laboratory where snout-vent length (SVL, with a digital calliper ± 0.02 mm) and weight (50 g spring scale ± 0.3 g) were measured every 7–10 days until parturition. Females were placed individually in terrariums $100 \times 20 \times 30$ cm (length x width x height) with sand as substrate and separated each other with opaque walls to avoid any interaction among individuals. During laboratory experiments, females were fed 4 times a week with *Tenebrio molitor* larvae and crickets (*Acheta domestica*) with a multivitamin and calcium supplement for reptiles. Females were provided with water *ad libitum*, a shelter and UVB light (Sylvania-Reptistar®) located 30 cm away from the lizards (*sensu* Lindgren 2004) during 3.5 h per day (from 12:30 to 4:00 pm, time in which lizards show the highest activity in nature).

All lizards were maintained in the same room, under bright fluorescent light tubes (TDL 36 W/541) that

mimicked daily light conditions in a cycle of 12 h light : 12 h dark. Ambient temperature was recorded every 5 min using data loggers (HOBO Onset Computer Corporation) located inside the terrarium, in order to control the mean temperature during the day and night, and during the treatments.

Pregnant females were randomly assigned by coin toss to one of the two temperature treatments until parturition, in order to compare the effects of temperature experienced by pregnant females and by developing embryos in: the reproductive success, the timing of parturition, and the offspring phenotypic traits (similar to methods performed by Shine in 1995 for the oviparous lizard *Nannoscincus maccoyi*). In the *Nest temperature treatment (Nest-T)* a group of pregnant females (N=16) was exposed to a thermal gradient from 17 to 25 °C; the 25 °C belongs to the 75% of the values of maximum temperature recorded in the field for a potential nest in the environment where *L. sarmientoi* were captured (see Results section). In the *Broad temperature treatment (Broad-T)* another group of pregnant females (N=14) was exposed to a thermal gradient from 17 to 45 °C to allow females to thermoregulate freely to achieve their preferred body temperature (T_{pref}).

The highest temperature of the thermal gradients of the *Nest-T* and *Broad-T* treatments was achieved by incandescent lamps (75 W) located at 40 and 20 cm respectively at one end of the terrarium. Treatments were carried out for 3.5 h per day, from 12:30 to 4:00 pm, when lizards showed the highest activity in nature. In the *Nest-T* treatment, lizards selected the body temperature by choosing from available temperatures (from 17 to 25 °C), whereas in the *Broad-T* treatment, lizards choose their T_{pref} from a broad range of temperatures (from 17 to 45 °C).

Recording T_{pref} During Experiments

The T_{pref} of pregnant females of both treatments, *Nest-T* and *Broad-T*, respectively, were measured for 2 h (from 1:30 to 3:30 pm) every 7 days the first 3 weeks and every 10 days at the end of the experiment (in order to minimise the animals' stress when they were manipulated). To do so, every female was connected to an ultra-thin catheter thermocouple (0.08 mm), fastened to the belly. In one of the females a simultaneous check of the temperature measured in the belly vs. the temperature measured in the cloaca was performed and the temperatures were not different (Paired *t* test: $t_{27}=0.45$, $P=0.659$). For this reason, all temperature measurements were made with the thermocouples fixed to the belly, in order to reduce the stress caused by connecting the thermocouple inside the cloaca of the pregnant females (as in this case females would not be able to defecate and, if required, would not be able to give birth, either).

Thermocouples were connected to a temperature Data Acquisition Module (USB-TC08, OMEGA) which recorded the body temperature every minute for 2 h. The T_{pref} for each lizard (from both the *Nest-T* and the *Broad-T* treatment) was estimated. The averages of the mean T_{pref} obtained from each treatment were used as the two experimental temperatures in the locomotor performance experiments of their newborns (see below).

In the last 5 days of the experiment (from March 5th to March 9th) the temperature of the *Nest-T* treatment was increased to allow females to thermoregulate at a higher temperature (maximum gradient temperature 40 °C, between 12:30 and 4:00 pm). This was done in order to accelerate the parturitions, which, until then, had not occurred in the females of the *Nest-T* treatment, and also in order to return the females and newborns of both treatments to their natural environment at the same time and before the end of the activity season.

T_b and T_{pref} Comparisons Between Pregnant and Non-pregnant Females

Activity body temperatures (T_b) in the field, and the T_{pref} measured after capture of females subjected to the *Broad-T* treatment (N=14) were compared with previous data of activity body temperatures, T_{pref} , and effectiveness of thermoregulation (E) obtained previously for non-pregnant adult females (N=34) during February 2007 and 2009. Based on these variables (T_b and T_{pref}) and the operative temperatures (T_e) the following indices for pregnant and non-pregnant females (*sensu* Hertz et al. 1993) were obtained: The index of the mean thermal quality of a habitat from an organism's perspective (d_e) was calculated as the mean of the absolute value of the differences between the T_e and the interquartile of the T_{pref} . The d_e index allowed us to estimate the availability of thermal microenvironments that are included in the interquartile of the T_{pref} . In addition, in order to measure the average extent to which *L. sarmientoi* experienced T_b outside the interquartile of the T_{pref} , the mean of the absolute values of the deviations of T_b from the interquartile of the T_{pref} of each lizard was obtained (d_b). The d_b index allowed us to estimate if the activity body temperatures of the lizard were included in the interquartile of the T_{pref} . The existence of active selection of the microhabitats by thermoregulation and the effectiveness of the thermoregulation was obtained as $E=1 - d_b/d_e$ which integrates the thermal resources that *L. sarmientoi* have in their natural microenvironments and their thermoregulation ability. The E index allowed us to estimate the existence of thermoregulation, corresponding E values close to 1, to an excellent thermoregulator; close to 0, to a thermoconformer or a passive thermoregulator;

and close to 0.50, to a moderate thermoregulator (Hertz et al. 1993; Bauwens et al. 1996; Medina et al. 2009; Iburgüengoytia et al. 2010).

Comparisons of Postpartum Females and Newborns From *Broad-T* and *Nest-T* Treatments

Relative Litter Weight

Terrariums were checked twice a day for newborns. After parturition, females were palpated to confirm that birth of all young was completed. Weight of neonates (Ohaus Explorer Electronic Balance ± 0.0001 g, Parsippany, New Jersey, USA) was obtained to calculate the relative litter weight, as the residuals of the regression between the natural logarithm (ln) of the total weight of neonates of the litter vs. ln mother weight after gave birth.

Body Condition

Body condition of postpartum females and offspring was calculated using the scaled mass index of each individual (postpartum female or newborn) as an estimator of the energy store or fat reserves. The scaled mass index of condition was calculated as: $M = M_i \times [SVL_0/SVL_i]^{b^{SMA}}$, where M_i and SVL_i are the body mass and SVL of individual i respectively, SVL_0 is the arithmetic mean SVL of the population, and b^{SMA} is estimated by the standardized major axis (SMA) from the regression of ln mass on ln SVL for the population (Peig and Green 2009, 2010). The b^{SMA} exponent was calculated using the package “lmodel2” (Legendre 2015) in R (R Core Team 2015).

Date of parturition of each female and developmental state and viability condition of neonates was also registered. Neonates were characterised as “viable” when the external phenotypic features are well developed (*sensu* Lemus et al. 1981) and exhibit good health conditions (absent of abnormalities and vigorous movements). Only the viable neonates that were fully developed and in good health conditions were subjected to the locomotor performance experiment.

Newborns Locomotor Performance

Locomotor performance of *L. sarmientoi* newborns was assessed between 24 and 48 h after birth (following Angilletta Jr. et al. 2002; Fernández et al. 2011) as a measure of body performance (Wapstra 2000). Newborns were removed from the maternal terrarium without previously feeding them, and were left in a quiet site before runs. In order to detect differences in the phenotype of newborns, they ran at the mean temperature selected by pregnant

females of the *Nest-T* treatment (25 °C), and also at the mean T_{pref} selected by pregnant females of the *Broad-T* treatment (34 °C), according to the results of this study. Those temperatures were achieved using a heater system comprised by a thermal water bath (with controlled temperature and thermostat) and a fan heater that maintained the ambient temperature to the experimental temperature. Newborns' body temperature before runs was reached by placing them in a sealed container (with holes on top to allow air entry) inside the thermal water bath (± 0.5 °C, 6 l) for 20 min, following Wapstra (2000) methodology.

Newborns were made to run in a wooden racetrack 1.15 m long, with cork as a substrate, a shelter at one end, and eight infrared photoreceptors held 0.15 m apart and linked to a computer. Running times between photoreceptors were utilized to calculate the running speed. Temperature experiments were made randomly: a group of newborns started running at 25 °C and another at 34 °C. Newborns ran at both temperatures (25 and 34 °C) the same day, with a resting minimum period of 4 h between experiments. Newborns were encouraged to run by gently touching their tail or the back of their legs, taking care not to interfere with the running speed. Lizards ran three times at each temperature experiment, with a resting period of 30 min between repetitions (Wang et al. 2014), and the fastest of the three speeds obtained by each lizard was considered for the analyses, following Shine (2004). The trials in which the newborn refused to run, stopped, or turned and ran the wrong way were excluded from the analysis. The speed for sprint runs (SR), considered as the fastest speed over the first 0.15 m of the racetrack, was obtained as an estimate of acceleration and the fright reaction of the lizard frequently observed in the field. Furthermore, the speed for long runs (LR), the fastest speed over the entire racetrack (1.05 m), was calculated as an index of the ability of the lizard to maintain speed along a relatively long distance, and represented the locomotor capability of the lizard to perform activities such as foraging, dispersion and evading predators.

Newborns Morphometric Measures

For all neonates, the SVL, inter-limb length, head length, head width, distance between knees, tail length and total length (SVL + tail length) were measured using a digital calliper (± 0.02 mm). After measuring them, offspring were provided with food and water *ad libitum*. Mothers with their offspring were released in good condition to the exact site of capture of each female, using data of the capture sites previously geo-referenced (GPS GARMIN) and photos of each microenvironment.

Statistical Analyses

We used the statistical software programs Sigma Stat 3.5®, SPSS 15.0®, Sigma Plot 10.0®, and R (R Core Team 2015). Assumptions of normality and homogeneity of variance were checked using Kolmogorov–Smirnov and Levene's Tests, respectively. Means were given with \pm standard error (\pm SE). In order to achieve the thermal source used for thermoregulation, the relationship between micro-environmental temperature variables and body temperature for pregnant and non-pregnant females, as well as the relationship between SVL and litter size for pregnant females, was analysed by simple Linear Regressions.

We used *t*-Test comparisons to analyse differences between postpartum females from the *Broad-T* and *Nest-T* treatments in the SVL, the relative litter weight and body condition, and to determine the differences between pregnant and non-pregnant females in the thermoregulation indexes (T_{pref} , T_b , d_e , d_b and E). When normality and/or homogeneity of variance failed, we used the non-parametric tests Mann–Whitney for comparison of medians of two independent samples, and Wilcoxon for the comparison of medians of repeated samples for two trials. Differences between paired sample means of the newborns speed between 25 and 34°C trials were analysed using Paired *t*-Test. Proportion of females that gave birth vs. total pregnant females and proportion of viable neonates vs. total of neonates were analysed with a Fisher's Exact Test.

Morphometric differences and body condition between offspring from *Broad-T* and *Nest-T* treatments were analysed using a Nested Analysis of Variance (ANOVA), considering the mother as the nested variable and the total length as the co-variable in the model (significant co-variable, $P < 0.05$ total length in all morphometric variables). In addition, differences in the speed for sprint and long runs between newborns from *Broad-T* and *Nest-T* treatments were analysed using a Nested ANOVA, considering the mother as the nested variable and the morphometric variables as co-variables when they were significant in the model (the only significant co-variable was inter-limb length, $P < 0.05$ for speed SR 34°C and LR 25°C).

Results

Operative Temperatures

The mean temperature for potential nests of an oviparous lizard was $12.27 \pm 0.28^\circ\text{C}$. The 75% interquartile of values of the maximum temperatures recorded in the potential nests were 24.82°C (Fig. 1a) and were used as the maximum temperature of the thermal gradient for the *Nest-T* treatment. The mean operative temperature for

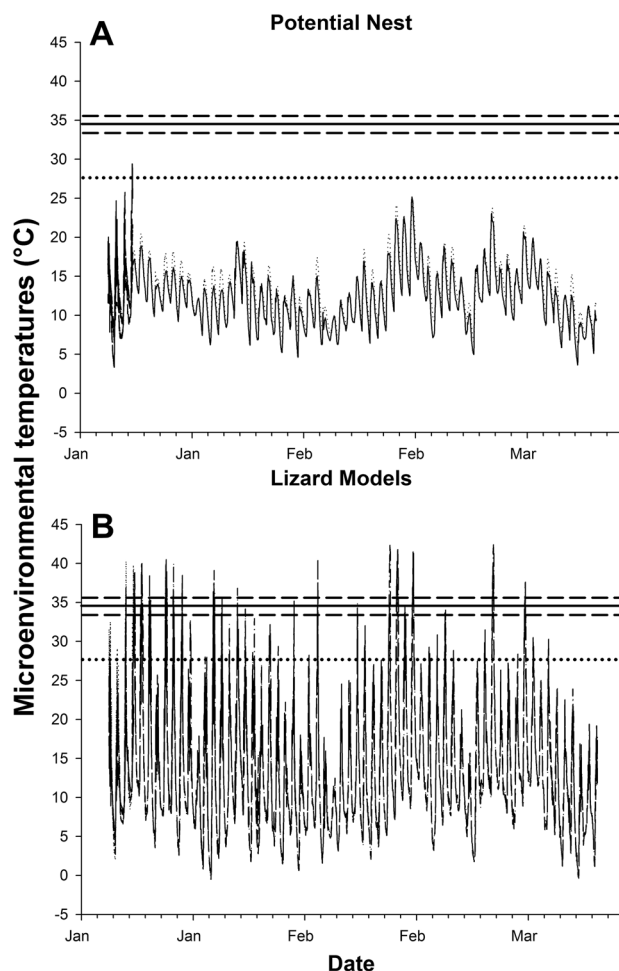


Fig. 1 Distribution of micro-environmental temperatures during the day and night throughout summer. **a** Data recorded by seven thermistors connected to four data loggers located in potential nests, and **b** operative temperatures recorded by six thermal models connected to three data loggers located in microenvironments where lizards thermoregulate. The mean body temperature recorded during captures (dotted line), the mean (solid line) and range (between dashed lines) of preferred body temperatures of pregnant females are indicated

lizard thermoregulation was $11.84 \pm 0.13^\circ\text{C}$ (from -0.51 to 42.65°C , Fig. 1b), recorded in different microenvironments during the day and night throughout summer.

Pregnant Females Experiments

Pregnant females showed a relationship between SVL and litter size (simple Linear Regressions: $F_{1,24} = 4.64$, $P = 0.042$, $r^2 = 0.17$; Mean SVL = 75.02 ± 0.83 mm, Median litter size = 4, from 2 to 5 offspring). Pregnant females of the *Broad-T* and *Nest-T* treatments did not show differences in the SVL (*t* test: $t_{28} = -0.67$; $P = 0.507$; Mean_{*Broad-T*} = 74.42 ± 3.96 mm, $N = 14$; Mean_{*Nest-T*} = 75.47 ± 4.56 mm, $N = 16$). During captivity of a total of 30 individuals collected, nine females increased

between 2.4 and 10.9% of weight, six females did not change their weight, and 15 females decreased between 1.2 and 13.3% of weight.

The mean T_{pref} of pregnant females subject to the *Broad-T* was 34.85 ± 0.16 °C (N=14) and the T_{pref} of females of the *Nest-T* treatment was 24.77 ± 0.12 °C (N=16). The ambient temperature during captivity was between 14.6 and 26.5 °C with a mean temperature of 19.6 ± 0.01 °C.

Most females of the *Broad-T* treatment gave birth before females of the *Nest-T* treatment: eleven females of the *Broad-T* treatment gave birth between February 13th and 26th, and three females gave birth in March 1st, 6th and 7th. Two females of the *Nest-T* treatment gave birth in February 9th and 17th, and eight females gave birth only after the temperature was raised at the end of the experiment to induce parturition, between March 5th and 9th. Considering the gestation period as captives (*sensu* Shine and Harlow 1993), females of the *Broad-T* treatment gave birth earlier than females of the *Nest-T* treatment (Mann–Whitney: $U=34.5$; $P=0.040$; $Median_{Broad-T} = 40.5$ days, N=14; $Median_{Nest-T} = 54.5$ days, N=10).

There were no differences in the relative litter weight and body condition of postpartum females between pregnant females of the *Broad-T* and *Nest-T* treatments (Table 1). All females of the *Broad-T* treatment gave birth at the end of the experiment, and all their newborns were born in good conditions. In contrast, the 62.5% of pregnant females of the *Nest-T* treatment gave birth at the end of the experiment, offspring which only the 22.2% were viable individuals (Table 1).

T_b and T_{pref} Comparisons Between Pregnant and Non-pregnant Females

There were no differences in the mean T_b or in the T_{pref} between pregnant and non-pregnant females (Table 2; Fig. 2). In contrast, standard deviation, coefficient of variation, range, minimum and maximum T_{pref} were higher in non-pregnant females (Table 2; Fig. 2). Both pregnant and non-pregnant females have an E index close to 0.50, which corresponds to moderate thermoregulators (Table 2).

Table 1 Mean of the relative litter weight and body condition of postpartum female, proportion of females that gave birth/total pregnant females, and the proportion of viable neonates/total neonates from the *Broad-T* and *Nest-T* treatments

	<i>Broad-T</i> treatment		<i>Nest-T</i> treatment		<i>t</i> Test or Fisher's exact test, and P value (P)
	Mean or median and proportion (%)	N	Mean or median and proportion (%)	N	
Relative litter weight (g)	0.05 ± 0.08	14	-0.07 ± 0.10	10	$t_{22} = 0.96$ (0.346)
Body condition of postpartum females	29.85 ± 1.109	14	30.35 ± 1.468	10	$t_{22} = -0.27$ (0.786)
Females that gave birth/ total pregnant females	14/14 = 100%		10/16 = 62.5%		(0.019)
Proportion of viable neonates/ total neonates	49/49 = 100%		8/36 = 22.2%		(<0.001)

Significant *P* value is highlighted

Table 2 Mean or medians of the following thermoregulation indexes obtained for each pregnant and non-pregnant female of *L. sarmientoi*: mean, standard deviation, coefficient of variation, range, minimum

and maximum preferred body temperature (T_{pref}), mean field body temperature (T_b), and the d_e , d_b and E indexes

Thermoregulation indexes	Pregnant females		Non-pregnant females		<i>t</i> Test or Mann–Whitney (<i>U</i>) and <i>P</i> value (<i>P</i>)
	Mean or median	N	Mean or median	N	
T_{pref}	34.57 ± 0.21 (33.49–35.85)	14	35.1 (26.26–37.81)	34	$U=282.00$ (0.324)
T_{pref} standard deviation	1.26 ± 0.08	14	2.80 (1.13–6.48)	34	$U=455.00$ (<0.001)
T_{pref} coefficient of variation	0.23 ± 0.01	14	1.15 (0.43–2.47)	34	$U=476.00$ (<0.001)
T_{pref} range	5.62 ± 0.36	14	11.76 ± 0.66	34	$U=434.00$ (<0.001)
T_{pref} minimum value	31.73 (28.57–33.06)	14	27.83 ± 0.70	34	$U=105.00$ (0.003)
T_{pref} maximum value	36.93 ± 0.16	14	39.58 ± 0.34	34	$U=431.00$ (<0.001)
T_b	27.80 ± 1.19	14	27.34 ± 0.75	34	$t_{46} = 0.32$ (0.753)
d_b	6.01 ± 1.23	14	6.20 ± 0.76	34	
d_e	12.69 ± 1.94		10.88 ± 0.50		
E	0.53		0.43		

Significant *P* value is highlighted

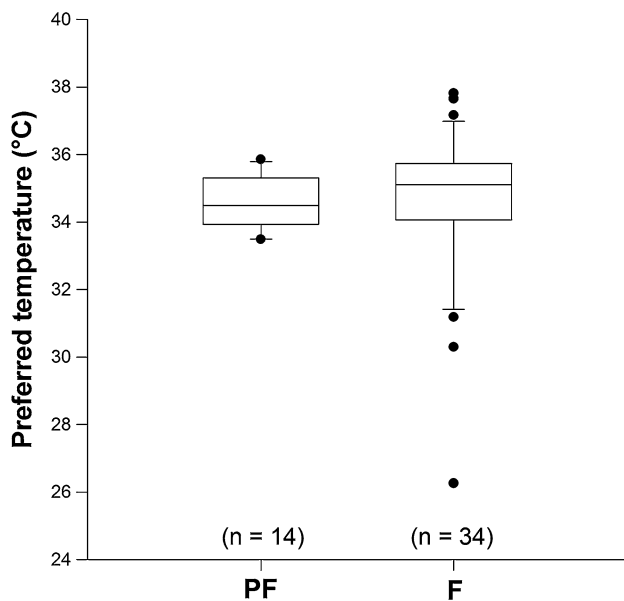


Fig. 2 Distribution of the preferred body temperatures for *L. sarmientoi* pregnant females (PF) and non-pregnant females (F). The 10, 25, 75 and 90% percentiles and the sample size (N) are indicated

Thermal Resource Used By Pregnant and Non-pregnant Females For Thermoregulation

Pregnant females showed a strong relationship between micro-environmental temperature variables and body temperatures (simple Linear Regressions: T_s , $F_{1,3} = 31.22$, $P < 0.001$, $r^2 = 0.72$; T_a , $F_{1,3} = 16.29$, $P = 0.002$, $r^2 = 0.58$), in contrast with non-pregnant females (T_s , $F_{1,32} = 3.78$, $P = 0.061$, $r^2 = 0.11$; T_a , $F_{1,32} = 2.49$, $P = 0.125$, $r^2 = 0.07$). Micro-environmental temperature data recorded during capture showed that pregnant females mainly used the thigmothermy and the heliothermy both at low and high temperatures. Non-pregnant females obtained the heat mainly by heliothermy at low temperatures, and at high temperatures thermoregulate at lower temperatures than substrate (Fig. 3). Body temperature of pregnant females can be better predicted by both micro-environmental variables (T_s and T_a , $r^2 > 0.57$) than non-pregnant females ($r^2 < 0.11$).

Comparisons of Newborns Born In The *Broad-T* and *Nest-T* Treatments

Newborns from the *Broad-T* treatment showed greater body condition, larger SVL, tail length, total length, interlimb length, distance between knees, head width, and they were also faster in sprint and long runs both at 25 and 34 °C than the newborns from the *Nest-T* treatment (Table 3; Fig. 4). Comparing the newborn speed within treatments, the newborns of the *Broad-T* treatment ran faster at 34 °C than at 25 °C, both in sprint runs (Wilcoxon Test:

$W = 772.00$; $N = 48$; $P < 0.001$; Fig. 4) as well as in long runs ($W = 1038.00$; $N = 48$; $P < 0.001$; Fig. 4). While the newborns of the *Nest-T* treatment did not show differences between speeds at 34 and 25 °C, both in sprint runs (Paired t -Test: $t_4 = 0.85$; $P = 0.441$; Fig. 4) as well as in long runs ($t_4 = 0.47$; $P = 0.664$; Fig. 4).

Discussion

In reptiles, the widely accepted idea is that viviparity has evolved from oviparity to provide embryos with more suitable temperatures than those they would experience in a nest in nature (Shine 2004; Webb et al. 2006). In *Liolaemus sarmientoi* pregnant females exposed to temperatures that simulated a nest in their natural environment (*Nest-T* treatment), selected temperatures near the maximum temperature available in the thermal gradient (24.77 ± 0.12 °C); while those pregnant females that were able to freely thermoregulate in a broad gradient of temperatures (*Broad-T* treatment), selected temperatures (34.85 ± 0.16 °C) near the T_{pref} for the species (34.4 ± 0.28 °C; Ibarguengoytía et al. 2010). Pregnant females exposed to the *Broad-T* gradient of temperatures had greater success during pregnancy, shorter gestation period, and their offspring showed better locomotor performance than those from females exposed to the *Nest-T* treatment. In addition, embryos developed at *Nest-T* treatment had a significant mortality (37.5% in *Nest-T* vs. 0% in *Broad-T*), abortions, and most neonates with abnormalities (nonviable neonates: 77.8% in *Nest-T* vs. 0% in *Broad-T*). In contrast, the temperature treatments did not affect the relative litter weight and body condition of postpartum females. *Liolaemus sarmientoi* pregnant females were exposed to the temperature treatments from a medium stage of embryonic development, in order to minimize the time in captivity which is related with greater stress and low embryo survivorship (J. B. Fernández, unpublished data). In this sense, it was reported that the early to medium period of embryonic development largely corresponds to the period with higher thermal sensitivity for lizards (i.e. of genus *Bassiana*, Shine and Elphick 2001). However, in view of the broad differences between the two temperature treatments in most of the variables analyzed in this study (except by the relative litter weight and postpartum body condition), we conclude that embryos may be particularly sensitive to low temperatures also late in development (as has been reported for *Vipera aspis*, Lourdais et al. 2004).

During pregnancy in nature, females of *L. sarmientoi* mainly used thigmothermy and heliothermy for thermoregulation, using a variety of thermal resources to reach their preferred body temperature. In contrast, non-pregnant females showed greater dispersion of body temperatures in relation to the micro-environmental temperatures for

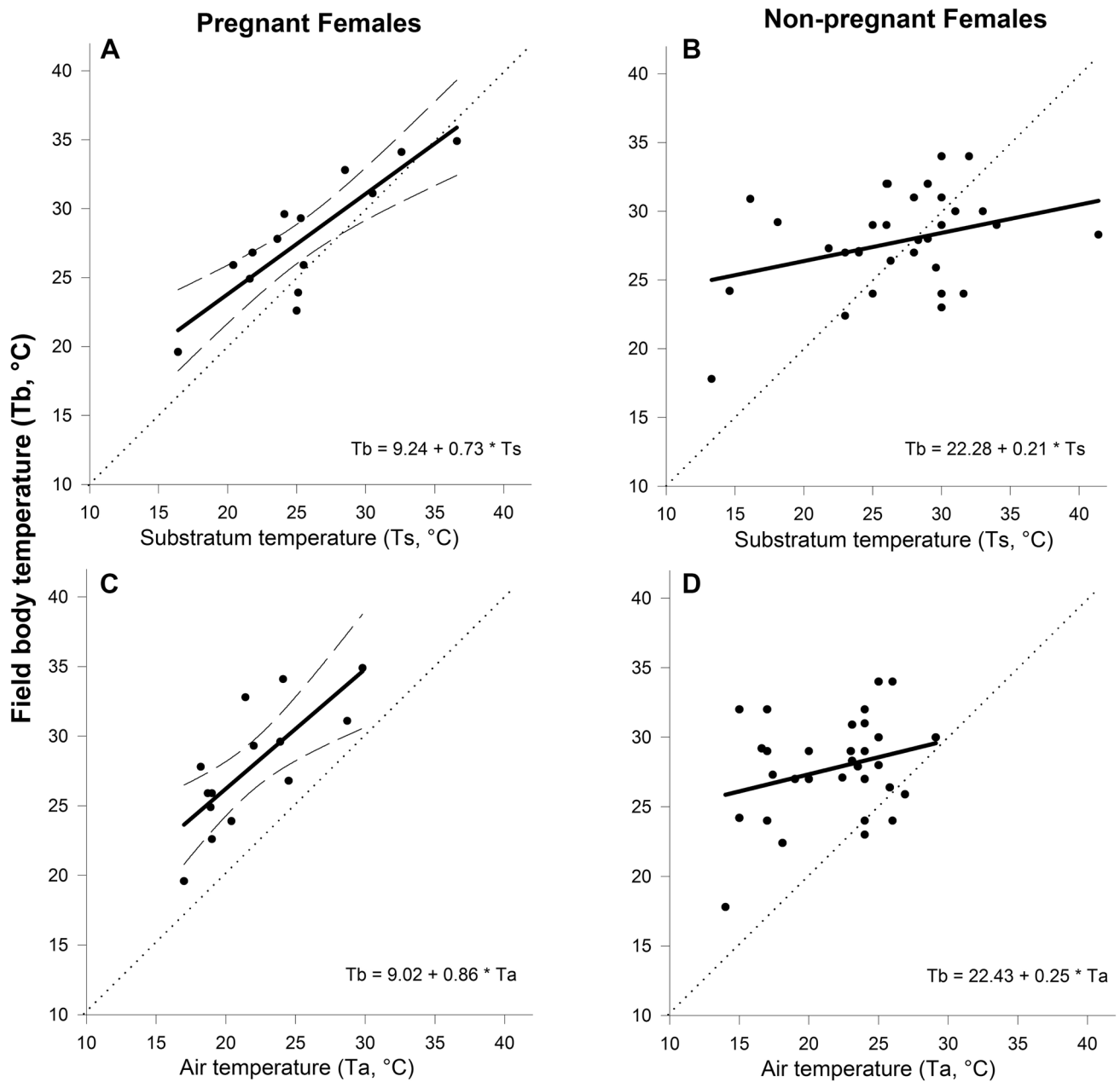


Fig. 3 Thermal resource used for thermoregulation of *L. sarmientoi* pregnant (N=14) and non-pregnant (N=34) females. **a** Body temperature vs. substratum temperature were determined by Linear regressions for pregnant females: $P < 0.001$, $r^2 = 0.72$ and **b** non-pregnant females: $P = 0.061$, $r^2 = 0.11$; **c** Body temperature vs. air tem-

perature were determined by Linear regressions for pregnant females: $P = 0.002$, $r^2 = 0.58$ and **d** non-pregnant females: $P = 0.125$, $r^2 = 0.07$. Linear regression (solid line), their 95% confidence intervals (dashed lines), and linear equation are indicated. Line of equality intersects the origin (dotted line)

thermoregulation, being less constrained and being able to exploit a wider array of temperatures. Therefore, pregnant females used a different thermoregulatory strategy to maintain their body temperature in a relatively narrow range of variation, probably related to the possibility of providing an adequate thermal environment for embryos and also to reduce the risk of predation and optimise energy (Lin et al. 2008). In this regard, it has been observed that most

L. sarmientoi non-pregnant females dare to bask in diverse thermoregulatory sites, in high rocky outcrops, distant from the shelter, unlike pregnant females who bask at a few centimetres from the entrance of their shelter (J. B. Fernández, unpublished observation). This thermoregulatory behaviour of pregnant females, in turn, can be related to their reduced locomotor ability, which makes the females less likely to refuge and escape from predation by running, as has been

Table 3 Means of body condition, morphometric variables (SVL, tail length, total length, inter-limb length, distance between knees, head length and head width), and speed for sprint runs (SR) and long runs (LR) at 25 and 34 °C of newborns from *Broad-T* and *Nest-T* treatments

Body condition, morphometric variables (mm) and speed of runs (m s ⁻¹)	Newborns from <i>Broad-T</i>		Newborns from <i>Nest-T</i>		Nested ANOVA (F) and P value (P)
	Mean	N	Mean	N	
Offspring body condition	2.56 ± 0.03	49	2.28 ± 0.11	11	F _{1,19} = 6.40 (0.020)
SVL (mm)	31.50 ± 0.17	49	30.22 ± 0.57	11	F _{1,18} = 18.58 (< 0.001)
Tail length (mm)	35.07 ± 0.48	48	31.92 ± 1.15	11	F _{1,18} = 116.80 (< 0.001)
Total length (mm)	66.10 ± 0.72	48	62.14 ± 1.68	11	F _{1,19} = 5.03 (0.037)
Inter-limb length (mm)	15.61 ± 0.19	49	14.42 ± 0.50	11	F _{1,18} = 6.87 (0.017)
Distance between knees (mm)	13.17 ± 0.08	49	12.70 ± 0.14	10	F _{1,17} = 6.66 (0.019)
Head length (mm)	8.45 ± 0.07	49	8.35 ± 0.22	11	F _{1,18} = 0.14 (0.714)
Head width (mm)	6.12 ± 0.04	49	5.76 ± 0.11	11	F _{1,18} = 6.92 (0.017)
Speed SR 25 °C (m s ⁻¹)	0.502 ± 0.04	49	0.115 ± 0.03	6	F _{1,16} = 9.98 (0.006)
Speed SR 34 °C (m s ⁻¹)	0.756 ± 0.05	48	0.098 ± 0.03	5	F _{1,14} = 18.43 (< 0.001)
Speed LR 25 °C (m s ⁻¹)	0.467 ± 0.02	49	0.125 ± 0.03	6	F _{1,15} = 23.11 (< 0.001)
Speed LR 34 °C (m s ⁻¹)	0.688 ± 0.03	48	0.117 ± 0.03	5	F _{1,15} = 18.44 (< 0.001)

Total length and inter-limb length were used as co-variables ($P < 0.05$) for comparisons between morphometric variables and for comparisons between running performance (only in SR at 34 °C and LR at 25 °C), respectively. Significant P value is highlighted

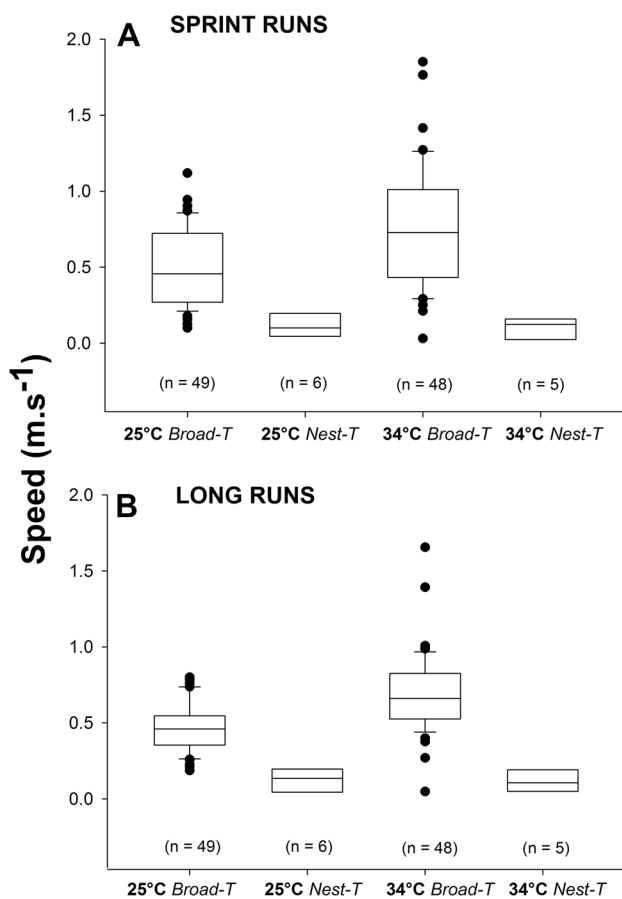


Fig. 4 Distribution of the speed for sprint (a) and long runs (b) at 25 and 34 °C for *L. sarmientoi* newborns from *Broad-T* and *Nest-T* treatments. The 10, 25, 75 and 90% percentiles and the sample size (N) are indicated

reported for other lizard species such as *Zootoca vivipara* (Van Damme et al. 1989), *Carinascincus microlepidotus* (Olsson et al. 2000), *Sceloporus occidentalis* (Sinervo et al. 1991) and *Liolaemus pictus* (Kubisch et al. 2011).

In addition, in some species of lizards and snakes, pregnant females prefer higher temperatures (Tu and Hutchison 1994; Charland 1995) and in other cases lower temperatures (Mathies and Andrews 1997; Ji et al. 2007b; Braña and Ji 2007; Lin et al. 2008; Li et al. 2009; Rodríguez-Díaz et al. 2010; Paranjpe et al. 2013) than non-pregnant females or males, in order to provide more suitable temperatures to embryos during development than regular environmental temperatures. Regarding *L. sarmientoi*, pregnant and non-pregnant females showed a similar mean T_{pref} , but pregnant T_{pref} distribution are in a narrower range of temperatures than non-pregnant females, and showed significant differences in standard deviation, coefficient of variation, range, and minimum and maximum T_{pref} . This possibility of pregnant females to precisely thermoregulate could allow embryos to develop in higher and more stable temperatures than they would experience in a nest in nature. The same occurs in other reptiles of cold climates (i.e. *Crotalus viridis*; Charland and Gregory 1990; *Phrynocephalus vlangalii*; Wang et al. 2014), and also in reptiles of tropical or temperate environments (i.e. *Acanthophis praelongus*; Webb et al. 2006 and *Gloydus brevicaudus*; Gao et al. 2010) in which the mean T_{pref} does not change while they are pregnant, but thermoregulate with less temperature variation than non-pregnant females.

In this sense, environmental temperatures, available for both *L. sarmientoi* pregnant females and for potential nests (Fig. 1), with large daily variations during the embryonic

developmental period probably explain the advantages of viviparity at this high latitude (51°S). Not only the mean but also the variance of temperatures during embryos development can significantly affect the embryogenesis and/or the phenotype of newborns in reptiles (Ji et al. 2003; Shine 2004; Webb et al. 2006). In this study, the experimental temperature range for pregnant *L. sarmientoi* (17–25 °C in *Nest-T* and 17–45 °C in *Broad-T*) had a remarkable effect on newborns in all morphometric variables (except the head length) and in the locomotor performance for sprint and long runs (both at 25 and 34 °C), in which the offspring of mothers with the possibility to reach the T_{pref} during pregnancy showed the highest values. Therefore, experimental thermal conditions for *L. sarmientoi* closer to what pregnant viviparous females experience in their natural environment through thermoregulation (variable and preferred temperatures) result in offspring with greater fitness. The same was reported in other studies with lizards (Shine 1995; Wapstra 2000; Li et al. 2009), snakes (O'Donnell and Arnold 2005; Webb et al. 2006; Gao et al. 2010) and turtles (Du and Ji 2003; Ji et al. 2003). Thus, females, and especially viviparous females, have the control of the phenotype of their embryos according to the environment in which they live, allowing the possibility of carrying out an early or late parturition to ensure greater fitness of their offspring. In cold-climates of high latitudes with short activity seasons and long brumation periods, an early birth before winter is more adaptive to ensure offspring survivorship (Rodríguez-Díaz et al. 2010; Wapstra et al. 2010; Wang et al. 2014). This pattern also avoids large numbers of *in utero* deaths, as has happened in *Sceloporus jarrovi* with induced prolonged gestation (Guillette et al. 1991) and in *L. sarmientoi* embryos from *Nest-T* treatment. Females of *L. sarmientoi* subjected to the *Broad-T* treatment gave birth before the females of the *Nest-T* treatment, 80% of which gave birth only after the temperature was increased at the end of the experiment in order to allow parturitions.

Cold climate environments are challenging for embryonic development and provide a selective advantage for any evolutionary adaptation to allow females to maintain appropriate temperatures for embryonic development, such as the evolution of egg retention in uterus and viviparity (Mell 1929; Shine 1995; Qualls and Andrews 1999). The two hypotheses that explain the evolution of viviparity in reptiles could be also applied in *L. sarmientoi*. Pregnant females are moderate thermoregulators that aim to achieve the preferred body temperature, which is higher than the mean temperature experienced in the natural environment. This supports the cold-climate hypothesis, since offspring of females exposed to temperatures similar to their environment were not properly developed or not born in time to grow enough before brumation, and, in turn, presented low locomotor performance. Likewise, regarding the maternal

manipulation hypothesis, although it is necessary another experimental design to strongly support this hypothesis, pregnant females of *L. sarmientoi* showed less variability in their preferred body temperature than non-pregnant females avoiding extreme temperatures, and their offspring showed greater locomotor performance than offspring developed at lower temperatures. In this sense, if pregnant females can thermoregulate freely (with both time and thermal possibilities during the day) they can adjust their preferred body temperature to an optimal temperature range for offspring development, giving the possibility for embryos to develop at higher and more favourable temperatures and in a more precise and stable temperature range than they would experience in a nest. Thus, the offspring are born exhibiting a suitable phenotype to survive, showing a good locomotor ability (important to avoid predators and to maximise foraging success), with an appropriate body size and weight, and being born early enough in the season to reach a suitable size to face the long brumation period.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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