



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at SciVerse ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Thermal relationships between body temperature and environment conditions set upper distributional limits on oviparous species

Marlin Medina^{a,*}, Alejandro Scolaro^b, Fausto Méndez-De la Cruz^c, Barry Sinervo^d, Nora Ibarquengoytía^{e,f}

^a CONICET, Departamento de Biología, Facultad de Ciencias Biológicas, Universidad Nacional de la Patagonia San Juan Bosco sede Esquel, Ruta 259 km 4, Esquel, Chubut 9200, Argentina

^b CENPAT—CONICET, Universidad Nacional de la Patagonia San Juan Bosco, Trelew, Chubut 9100, Argentina

^c Laboratorio de Herpetología, Instituto de Biología, Universidad Nacional Autónoma de México, D.F. 04510, México

^d Department of Ecology and Evolutionary Biology, Earth and Marine Sciences Building University of California, Santa Cruz, CA 95064, USA

^e Departamento de Zoología, Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, Quintral 1250, San Carlos de Bariloche, Río Negro 8400, Argentina

^f INIBIOMA—CONICET, Universidad Nacional del Comahue, Quintral 1250, San Carlos de Bariloche, Río Negro 8400, Argentina

ARTICLE INFO

Article history:

Received 4 May 2011

Accepted 18 September 2011

Available online 22 September 2011

Keywords:

Squamata

Oviparity

Viviparity

Thermoregulation

Cold climate

Liolaemus

ABSTRACT

We determined the thermal biology of the oviparous *Liolaemus boulengeri* and the viviparous *Liolaemus lineomaculatus* populations localised at high and low latitude sites in Patagonia, Argentina. We present data of body temperatures in the field (T_b) and preferred temperature in the laboratory (T_{pref}), micro-environmental and operative temperatures and the effectiveness of thermoregulation. *Liolaemus boulengeri* and *L. lineomaculatus* choose different heat sources for active selection of suitable thermal micro-environments for thermoregulation, and the oviparous *L. boulengeri* is a more effective thermoregulator ($E=0.55$) than the viviparous *L. lineomaculatus* ($E=0.43$). Even when *L. boulengeri* is a better thermoregulator and both species show identical timing in the reproductive cycles, there are constraint factors that impose limitations on the southernmost distribution of the oviparous *L. boulengeri*.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

The relationship between thermoregulation and life history governs the course of adaptation in variable environments (Angilletta, 2009). Several lizard species are able to precisely thermoregulate in narrow ranges of temperatures (Pearson, 1954; Hertz et al., 1993; Zug et al., 2001). However, when heat sources or thermal microhabitats are scarce or ephemeral, compensatory changes can occur in behaviour and/or physiology (Niewiarowski, 2001) to maintain body temperature at a relatively high and constant level (Pearson, 1954; Hill, 1980; Zug et al., 2001). But, under thermal restrictions the costs of thermoregulation competes with the energy allocated to feed and to reproduce, and increases the risk of predation (Avery, 1978; Huey and Slatkin, 1976; Hertz and Huey, 1981; Sears, 2005).

For example, conspicuous differences exist between higher-elevation lizards regarding the extent of thermoregulation and activity period (Marquet et al., 1989). At high elevations, in tropical areas, lizards such as *Liolaemus signifer* from Perú

(4300 m; Pearson, 1954) or *L. huacahuasicus* from the Cumbres Calchaquíes, Tucumán, Argentina (4000 m; Halloy and Laurent, 1988) compensate for the low air temperatures (12 °C, weather stations records) by basking and taking advantage of the high solar radiation fluxes (Pearson, 1954) or by prolonging the activity period (Carothers et al., 1989; Marquet et al., 1989). In contrast, lizards in warmer climates typically avoid sunny patches when they achieve temperatures that are close to maximal body temperatures and are inactive at midday (Hertz et al., 1993; Sinervo et al., 2010).

However, effective thermoregulation becomes uncertain at high latitudes in Patagonia probably because of short daily and seasonal activity periods, combined with low air temperatures and the almost constant high wind intensity from the west (Paruelo et al., 1998; Ibarquengoytía et al., 2010). Species such as *L. bibronii* (43 and 46°S; Medina et al., 2009), *L. sarmientoi* and *L. magellanicus* (53°S, Ibarquengoytía et al., 2010) exhibit the lowest T_b recorded for 55 populations belonging to 33 species of the genus *Liolaemus*, and behave as thermoconformers or poor thermoregulators (sensu Hertz et al., 1993; Medina et al., 2009; Ibarquengoytía et al., 2010).

The main objective of this study was to explore the intra- and interspecific differences in field body temperature (T_b), preferred

* Corresponding autor. Tel./fax: +54 2945 450807x13.

E-mail address: marlinmedina74@gmail.com (M. Medina).

temperatures in laboratory (T_{pref}) and the effectiveness of thermoregulation (E , sensu Hertz et al., 1993) of two populations of an oviparous *L. boulengeri* and two of the viviparous *L. lineomaculatus* localised at different latitudes and altitudes. These two species were chosen, for comparative purposes, to complete previous studies on thermal biology on liolaemids from the southernmost distributions in Patagonia, such as those of *L. bibronii* (Medina et al., 2009), *L. magellanicus* and *L. sarmientoi* (Ibargüengoytía et al., 2010). The oviparous species *Liolaemus boulengeri*, included in the *chilliensis* group, inhabits the Monte and Steppe, and the high Andean grasslands environments, and is common at elevations up to 900 m asl in the Andean valleys from Mendoza Province (36°S; Cei, 1986; Scolaro, 2005) to the coast line of Santa Cruz Province (50°S; Scolaro, 2005; Morando et al., 2007; Pincheira-Donoso et al., 2008). The viviparous *L. lineomaculatus* belongs to the *signifer* group, and it inhabits from the north-west of Patagonia in the Neuquén Province (39°S) at elevations up to 900 m asl to the high Andean grasslands in Santa Cruz Province, where it is syntopic with the southernmost lizard of the world, *Liolaemus magellanicus* (51°S; Cei, 1986; Scolaro, 2005). Even though, *Liolaemus boulengeri* and *L. lineomaculatus* belong to two different phylogenetic groups and are representative of the oviparous and viviparous modes of reproduction, they are syntopic in part of their distribution and show similar reproductive traits such as timing of reproduction, clutch size and size at sexual maturity (Medina and Ibargüengoytía, 2010). So, our main question is whether thermal physiology and behaviour compensates for differences in body size, reproductive mode and genetic load.

2. Materials and Methods

2.1. Study area and materials

Two populations of *L. boulengeri* and two populations of *L. lineomaculatus* were studied in Patagonia, Argentina. In Esquel (Chubut Province), *L. boulengeri* was captured at a low elevation (43°01'S, 70°47'W, 626 m asl) and *L. lineomaculatus* at a high elevation (42°52'S, 71°12'W, 1400 m asl). In Perito Moreno (Santa Cruz Province) both species were captured in syntopy (46°37'S, 71°15'W, 263 m asl). Lizards (in Esquel: *L. boulengeri*, $N=98$; *L. lineomaculatus*, $N=76$; in Perito Moreno: *L. boulengeri*, $N=62$; *L. lineomaculatus*, $N=86$) were caught by hand or noose only when active (9 to 18 h), outside the burrows or performing any behaviour related to feeding and/or breeding activities, from February 2005 to March 2008.

2.2. Air temperatures and illuminance conditions of localities

Air temperatures and illuminance were recorded on the ground every 30 min during the activity season (from September to May) in the three localities. Data loggers were placed (Hobo Pendant, Temperature and Illuminance Data Logger) in similar conditions and exposed to solar radiation throughout the day.

2.3. Micro-environmental conditions at capture sites

In order to determine the main heat source used by the lizards (thigmothermy vs heliothermy) the substratum temperature in the sun (T_{ss}) and in the shade (T_{ssh}), and the air temperature at 1 cm above the ground (T_{a}) were recorded at each capture site, hereafter called micro-environmental temperatures. Temperatures were registered using thermocouples for substrate TES TP-K03 and air TES TP-K02, respectively, and they were connected to a TES 1302 thermometer (TES Electrical Electronic Corp., Taipei, Taiwan, ± 0.03 °C). Wind velocity (Turbo meter, ± 0.1 m/s), humidity

(Micro-meteorological station, Lutron LM-8000) and illuminance (Luximeter Extech model 401025, \pm lux) were also recorded at capture microsites. Time of day at capture was also recorded.

2.4. Determination of operative temperatures

Operative temperatures (T_e , sensu Bakken, 1992; Hertz, 1992) were recorded to describe the “null” distribution of body temperatures (T_b) expected in non-regulating animals integrating biophysical and morphological factors that influence an ectotherm's T_b . The appropriate model was designed using simultaneous comparisons of the body temperatures (T_b) of live lizards with that of copper models of varying sizes and colours. To choose the best model catheter probes TES TP-K01 were used simultaneously to register the temperatures of both the models and the lizards. The calibration experiments were conducted during 3 consecutive hours with half of the time in full sun and the other half in the shade. The best model was chosen by comparing the R^2 obtained from the correlation between each model and T_b . The best-fit model corresponded to a flat-black copper cylinder of 90 mm long \times 20 mm wide \times 2 mm thick, sealed at the ends. The correlation between T_b and T_{model} was significant (Lineal Regression, $F_{1, 50}=428.56$, $R^2=0.897$, $P<0.001$) and the R^2 range was from 0.87 to 0.97 (± 1 °C), considered acceptable in numerous previous studies (Beaupre, 1995; Dorcas et al., 1997; Dzialowski, 2005; Angilletta, 2009).

2.5. Lizards body temperatures

Body temperature (sensu Pough and Gans, 1982) was taken using a catheter probe TES TP-K01 (1.62 mm diameter) introduced ca. 1 cm inside the cloaca. Individuals were handled by head to avoid heat transfer and temperature was recorded within 10 s of handling.

2.6. Laboratory protocol and experiments

Experiments were performed 3 to 7 days after capture of lizards from Esquel (*L. boulengeri*, $N=44$; *L. lineomaculatus*, $N=63$) and from Perito Moreno (*L. boulengeri*, $N=46$; *L. lineomaculatus*, $N=69$) to obtain a preferred body temperature (T_{pref}). Lizards were placed individually in open-top terrarium (200 \times 45 \times 20 cm) each with a sand floor and a thermal gradient produced by a line of four infra-red lamps above (one of 250 W, two of 150 W and one of 100 W). The lamps were adjusted to different heights above the terrarium to make a linear substratum gradient from 15° to 65 °C. Temperatures were taken using ultra-thin (1 mm) catheter thermocouples located approximately 10 mm inside the cloaca and taped to the base of the lizard's tail to prevent the thermocouple from being dislodged during the experiment. The temperature of each lizard was obtained every 10 min for 5 h, by connecting the thermocouple to a TES 1302 thermometer (TES Electrical Electronic Corp., Taipei, Taiwan, ± 0.01 °C). The duration of the experiments was chosen considering the time during which *L. boulengeri* and *L. lineomaculatus* reach their preferred temperature during thermoregulation trials. The mean T_{pref} and the minimum and maximum set-point temperatures (T_{set} , the temperatures bounding the interquartile range, the middle 50% of the observations, for each lizard) were obtained for each lizard and were used to estimate the range and mean T_{pref} for each population and for the species.

2.7. Effectiveness of thermoregulation

The effectiveness of temperature regulation: $E=1-(\text{Mean } d_b/\text{Mean } d_e)$ was obtained. The d_b was calculated as the mean of the

absolute values obtained from the deviations of T_b from T_{set} of each individual and represents the average degree to which *L. boulengeri* and *L. lineomaculatus* experienced T_b outside the set-point range. Low values of d_b mean high accuracy in regulating temperature and high values of d_b , low accuracy (Hertz et al., 1993). The index d_e was calculated as the deviation of mean T_e from T_{set} , and indicates the average thermal quality of a habitat from the organism's perspective. Habitats with d_e equal to zero are thermally ideal, while habitats with high d_e are of lower thermal quality (Hertz et al., 1993). Thus, thermoregulation is considered effective when E is close to 1, thermoconforming when E is close to 0, and moderately effective if E is close to 0.50 (Hertz et al., 1993; Bauwens et al., 1996; also see an example for *Liolaemus* in Medina et al., 2009; Gutiérrez et al., 2010).

The snout-vent length (SVL; Vernier calliper, ± 0.1 mm) and body mass (BW, 10-g Pesola spring scale ± 0.5 g) were registered for each lizard. Reproductive condition and sex were determined for each individual by autopsy and histology of the gonads. This information was taken from a previous study (Medina and Ibagüengoytia, 2010).

2.8. Statistical analysis

We used the statistical software programs Sigma Stat 3.5[®], SPSS 11.0[®], Sigma Plot 10.0[®] for statistical analysis. The dependence between variables was analysed by simple, linear multiple or stepwise multiple regressions. The differences between the mean values of two samples were analysed using paired and unpaired *t*-tests. For more than two samples, we used a one way analysis of variance for independent samples or repeated samples and the Holm–Sidak test as a *posteriori* test, for related samples. The assumptions of normality and homogeneity of variance for parametric procedures were checked using Kolmogorov–Smirnov and Levene's tests, respectively.

When the assumptions of normality or homogeneity of variance were not met, we used equivalent nonparametric tests such as Mann–Whitney for comparison of means of two independent samples. For the comparison of means of repeated samples for two or three trials, we used Wilcoxon or Friedman repeated measures analysis of variance on ranks and the Tukey HSD test as a *posteriori* test, respectively. The significance level used for all statistical tests was $P < 0.05$ (Sokal and Rohlf, 1969; Norusis, 1986).

3. Results

3.1. Air temperature, illuminance and operative temperatures

In Esquel at 626 m asl mean monthly environmental temperatures (18.98 °C) and illuminance (290046.1 lx) were higher than

Table 2

Comparisons among mean \pm standard error (paired *t*-test, *t*) or median (Wilcoxon, *Z*) of body (T_b) and micro-environmental temperatures (°C): substratum temperature in the sun (T_{ss}) and in the shade (T_{ssh}), air temperature at 1 cm (T_a) and operative temperature (T_e) within Esquel and Perito Moreno populations of *L. boulengeri* and *L. lineomaculatus*. Sample size (*N*).

<i>Liolaemus boulengeri</i>					
	T_{ss}	T_{ssh}	T_a	T_e	
Esquel					
$T_{ss} = 37.17 \pm 1.09$ (58)					
$T_{ssh} = 24.38 \pm 0.55$ (58)	$Z = 5.77^*$				
$T_a = 25.39 \pm 0.55$ (99)	$t = -13.96^*$	$t = -7.92^*$			
$T_e = 23.65 \pm 0.77$ (44)	$Z = -5.72^*$	$t = -0.62$	$Z = 4.42^*$		
$T_b = 28.92 \pm 0.5$ (98)	$t = -6.07^*$	$t = -10.63^*$	$t = 5.96^*$	$t = 9.14^*$	
Perito Moreno					
$T_{ss} = 32.6 \pm 1.05$ (60)					
$T_{ssh} = 20.39 \pm 0.65$ (59)	$t = 15.71^*$				
$T_a = 23.73 \pm 0.68$ (60)	$t = 13.61^*$	$Z = 5.37^*$			
$T_e = 24.20 \pm 0.54$ (46)	$Z = 5.87^*$	$t = 7.29^*$	$t = -1.08$		
$T_b = 27.76 \pm 0.54$ (60)	$t = -5.47^*$	$t = 12.17^*$	$t = 6.47^*$	$t = 8.44^*$	
<i>Liolaemus lineomaculatus</i>					
	T_{ss}	T_{ssh}	T_a	T_e	
Esquel					
$T_{ss} = 27.71 \pm 0.99$ (45)					
$T_{ssh} = 15.76 \pm 0.85$ (45)	$t = -11.6^*$				
$T_a = 19.31 \pm 0.78$ (50)	$t = 10.66^*$	$t = 4.49^*$			
$T_e = 19.92 \pm 0.98$ (60)	$t = -11.3^*$	$t = 0.98$	$Z = 3.94^*$		
$T_b = 26.19 \pm 0.63$ (62)	$t = -1.52$	$t = -7.30^*$	$t = -7.31^*$	$Z = -3.94^*$	
Perito Moreno					
$T_{ss} = 29.89 \pm 0.53$ (89)					
$T_{ssh} = 20.41 \pm 0.41$ (89)	$t = -21.76^*$				
$T_a = 21.81 \pm 0.37$ (89)	$t = 20.77^*$	$Z = -4.65^*$			
$T_e = 22.4 \pm 0.44$ (73)	$t = -16.11^*$	$Z = -5.65^*$	$Z = -0.74$		
$T_b = 26.15 \pm 0.41$ (90)	$Z = 6.08^*$	$t = 14.65^*$	$t = 9.79^*$	$t = 9.43^*$	

* Indicated as significance $P < 0.05$.

Table 1

Comparisons between mean \pm standard error (*t*-test, *t*) or median (Mann–Whitney) of micro-environmental temperatures (°C): substratum temperature in the sun (T_{ss}) and in the shade (T_{ssh}), air temperature at 1 cm (T_a), operative temperatures (T_e), wind velocity (kmh^{-1}), humidity and illuminance between Esquel and Perito Moreno populations of *L. boulengeri* and *L. lineomaculatus*. Sample size (*N*), statistics and significance (*P*) and elevation at capture sites are indicated.

Micro-environmental variables	ESQUEL	PERITO MORENO	Statistic	<i>P</i>
<i>Liolaemus boulengeri</i> (oviparous)				
Elevation (m)	626	263		
T_{ss}	37.17 ± 1.09 (58)	32.6 ± 1.05 (60)	$t = 4016.5$	< 0.002
T_{ssh}	24.38 ± 0.55 (58)	20.39 ± 0.65 (59)	$t = 4.68$	< 0.002
T_a	25.39 ± 0.55 (99)	23.73 ± 0.68 (60)	$t = 1.88$	> 0.062
T_e	23.33 ± 0.76 (120)	24.36 ± 0.62 (120)	$T = 13743$	> 0.36
Wind velocity	7.1 (79)	16.3 (69)	$T = 5337$	< 0.001
Humidity	18.7 (57)	26.8 (57)	$T = 4232.5$	< 0.001
Illuminance	765 (65)	242 (58)	$T = 2849$	< 0.05
<i>Liolaemus lineomaculatus</i> (viviparous)				
Elevation (m)	1400	263		
T_{ss}	27.71 ± 0.99 (45)	29.89 ± 0.53 (89)	$T = 1889$	> 0.09
T_{ssh}	15.76 ± 0.85 (45)	20.41 ± 0.41 (89)	$T = 1083$	< 0.001
T_a	19.31 ± 0.78 (50)	21.81 ± 0.37 (89)	$T = 1845.5$	< 0.001
T_e	15.94 ± 0.8 (105)	24.09 ± 0.61 (132)	$T = 8376$	< 0.001
Wind velocity	4.45 (56)	33.1 (87)	$T = 2990.5$	< 0.005
Humidity	24.3 (53)	31 (83)	$T = 3176.5$	< 0.005
Illuminance	1384 (33)	833 (73)	$T = 2429$	< 0.001

in Esquel at 1400 m asl (13.71 °C; 226245.4 lx) and in Perito Moreno at 263 m asl (14.25 °C, 76236.2 lx, Friedman Repeated Measures Analysis of Variance on Ranks, temperature: $\chi^2=80.63$, $P<0.001$; Tukey Test, Esquel 626 m vs Esquel 1400 m: $q=11.2$; Esquel 626 m vs Perito Moreno 263 m: $q=10.8$; $P<0.05$; illuminance: $\chi^2=165.92$, $P<0.001$; Tukey Test, Esquel 626 m vs Esquel 1400 m: $q=5.14$; Esquel 626 m vs Perito Moreno 263 m: $q=12.56$, $P<0.05$). Operative temperatures (T_e) of *L. boulengeri* from Esquel and Perito Moreno localities were similar, while T_e of *L. lineomaculatus* from the Perito Moreno locality was higher than in Esquel (Table 1).

3.2. Determination of main heat source: relationship between micro-environmental and body temperatures

The results of comparisons between micro-environmental variables, T_{ss} , T_{ssh} and T_a at 1 cm above the ground, T_e , wind velocity, humidity and illuminance between Esquel and Perito Moreno populations of *L. boulengeri* and *L. lineomaculatus* are shown in Table 1. *Liolaemus boulengeri* and *L. lineomaculatus* relate differently to the micro-environmental temperatures at the capture site. The T_b of both populations of *L. boulengeri* and *L. lineomaculatus* was higher than the air temperature at 1 cm above the ground, the substrate temperature in the shade and the operative

temperature (Table 2). In *L. boulengeri* the T_b was lower than the substratum temperature in the sun (T_{ss}) in both populations, while in *L. lineomaculatus* T_b was lower than T_{ss} only in Perito Moreno (Table 2). These trends were also significant throughout the day and most confidence intervals between T_b and micro-environmental variables are significant ($P<0.05$, Figs. 1 and 2).

In Esquel *L. boulengeri* T_b showed a significant and positive relationship with T_a at 1 cm above the ground, while *L. lineomaculatus* showed a significant and positive relationship with T_{ss} (Stepwise Regression, $F_{1, 33}=15.43$, $P<0.001$). In Perito Moreno *L. boulengeri* T_b showed a significant and positive relationship with T_a and a negative relationship with the wind velocity (Stepwise Regression, $F_{T_a, 1.54}=50.33$; $F_{Wind, 2.53}=35.07$, $P<0.001$), and *L. lineomaculatus* showed a significant and positive relationship with both the substrate temperature in the sun (T_{ss}) and in the shade (T_{ssh} , Stepwise Regression, $F_{T_{ss}, 1.77}=34.25$; $F_{T_{ssh}, 1.76}=20.15$, $P<0.001$), even when T_a , wind velocity and illuminance were included in the analysis.

3.3. Relationship between body temperature in the field, preferred body temperature in the laboratory and reproductive state

Liolaemus boulengeri and *L. lineomaculatus* did not show intraspecific differences in T_b between sites (Table 3). Consequently, the

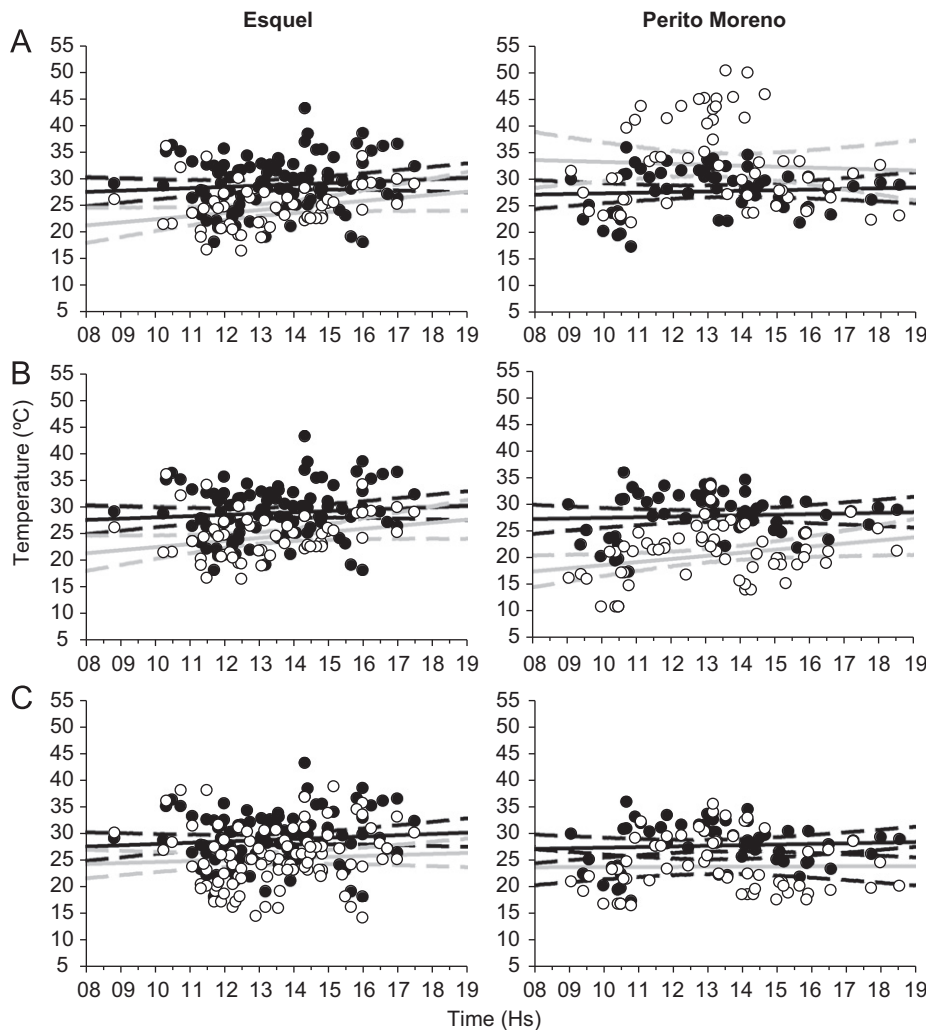


Fig. 1. Linear regressions (solid lines) and 95% confidence intervals (dashed lines) of body temperature (black lines and circles) and micro-environmental temperatures (grey lines and white circles): substrate in the sun (T_{ss} , panel A), substrate in the shade (T_{ssh} , panel B) and air temperature (T_a , panel C) vs. time (h) of *L. boulengeri* from Esquel and Perito Moreno.

mean values of the T_{bS} of both populations for each species were used for the analysis in this section (28.5 ± 0.37 and 26.0 ± 0.37 °C, respectively).

The T_{bS} of *L. boulengeri* were significantly lower in egg-carrying females (Mean = 27.47 ± 1.36 °C) than in non-egg-carrying females (ANCOVA, $F_{1,49} = 4.77$, $P < 0.05$; Mean_{non-egg-carrying} = 30.95 ± 0.68 °C). In *L. lineomaculatus*, T_b did not show differences between pregnant and non-pregnant females (ANCOVA, $F_{1, 52} = 0.04$, $P > 0.70$).

The T_{pref} of *L. boulengeri* was not significantly different between populations (ANOVA, $F_{1, 90} = 0.64$, $P > 0.42$; Mean_{Esquel} = 33.82 ± 0.17 ; Mean_{P. Moreno} = 32.92 ± 0.2), but in *L. lineomaculatus* the T_{pref} of lizards from Perito Moreno (Mean = 31.99 ± 0.46) was lower than in the individuals from Esquel (ANOVA, $F_{1, 133} = 16.34$, $P < 0.001$; Mean_{Esquel} = 34.5 ± 0.39 ; Table 3). From here on the mean T_{pref} for both populations was used for *L. boulengeri* (32.8 °C) and *L. lineomaculatus* (33.13 °C).

Conversely to the patterns noted above for T_b , *L. boulengeri* did not show differences between T_{pref} of egg-carrying and non-egg-carrying females (t -test, $t_{47} = 1.67$, $P > 0.1$), while in *L. lineomaculatus* the T_{pref} of pregnant females was significantly lower than in non-pregnant females (t -test, $t_{52} = 2.35$, $P < 0.049$).

The T_b was lower than the T_{pref} in both species (Paired t -test, $t_{L. boulengeri, 90} = 5.44$; $t_{L. lineomaculatus, 125} = 13.73$, $P < 0.001$).

3.4. Preferred body temperature and effectiveness of temperature regulation in the populations

In *L. boulengeri*, the lower and upper $T_{set\ point}$ was similar between populations (Table 3). In both populations most of the T_{bS} (Esquel: 49%; P. Moreno: 54.35%) were lower than the minimum T_{set} , and a smaller proportion of T_{bS} were higher than the maximum T_{set} (Esquel: 18%; P. Moreno: 2.15%; Fig. 3).

In *L. lineomaculatus* the lower and upper $T_{set\ point}$ were higher in the Esquel population than in Perito Moreno (Table 3). In both populations a greater proportion of T_{bS} (Esquel: 79.25%, P. Moreno: 69.86%) were lower than the lower- $T_{set\ points}$, and only in the P. Moreno population were 2.74% of T_{bS} higher than the upper $T_{set\ point}$ (Fig. 3).

The index d_b (the mean of the absolute values obtained from the deviations of T_b from T_{set} of each individual) was similar between populations of *L. boulengeri* and *L. lineomaculatus*. The index d_e (the deviation of mean T_e from T_{set}) in both populations of *L. boulengeri* did not show significant differences, but in *L. lineomaculatus* the index d_e was higher in Esquel at 1400 m asl than in the Perito Moreno site (Table 3).

The effectiveness of thermoregulation ($E = 1 - (\text{Mean } d_b / \text{Mean } d_e)$) was higher in both populations of *L. boulengeri* ($E_{Esquel} = 0.59$; $E_{P. Moreno} = 0.50$) than in populations of *L. lineomaculatus* ($E_{Esquel} = 0.45$; $E_{P. Moreno} = 0.41$; Table 3).

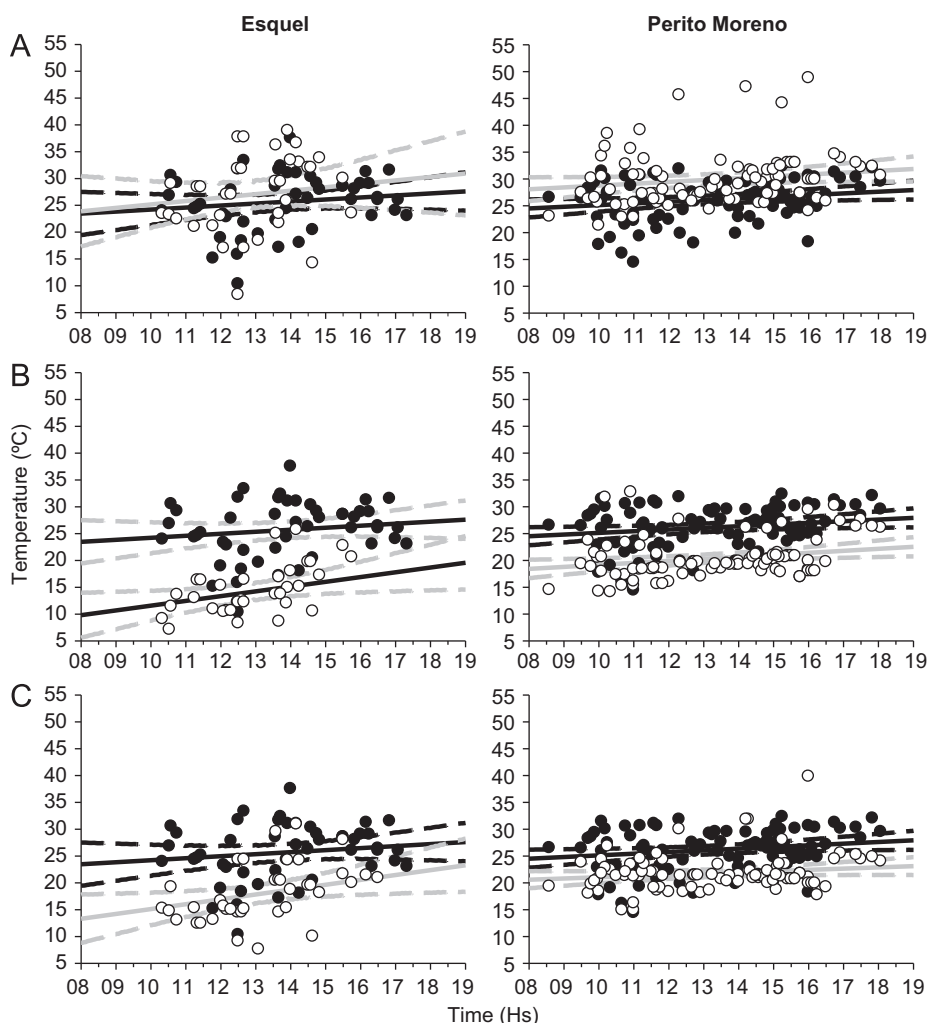


Fig. 2. Linear regressions (solid lines) and 95% confidence intervals (dashed lines) of body temperature (black lines and circles) and micro-environmental temperatures (grey lines and white circles): substrate in the sun (T_{ss} , panel A), substrate in the shade (T_{ssh} , panel B) and air temperature (T_a , panel C) vs. time (h) of *L. lineomaculatus* from Esquel and Perito Moreno.

Table 3
Comparisons between Esquel and Perito Moreno in mean \pm standard error (ANCOVA and ANOVA) or median (Mann–Whitney) body and preferred body temperatures (T_b and T_{pref} , °C), set-point temperature range (lower and upper, °C) and absolute values obtained from the deviations of T_b from T_{set} of each individual (d_b), index of the average thermal quality of an habitat (d_e) and the effectiveness of temperature regulation (E). Sample size (N) and significance $P < 0.05$ are indicated.

	T_b	T_{pref}	T_{set}		d_b	d_e	E
			Lower	Upper			
<i>Liolaemus boulengeri</i>							
Esquel	28.9 \pm 0.5 (98)	33.8 \pm 0.17 (45)	30.8 \pm 0.9 (44)	35.9 \pm 0.6 (44)	3.3 \pm 0.5 (44)	8.2	0.59
Perito Moreno	27.8 \pm 0.54 (60)	32.9 \pm 0.2 (46)	29.3 \pm 0.9 (46)	36.9 \pm 0.3 (45)	3.1 \pm 0.5 (46)	6.2	0.5
Comparisons	$F_{1,158}=1.47$ $P=0.23$	$F_{1,90}=0.64$ $P=0.42$	$T=2229$ $P=0.07$	$T=1794.5$ $P=0.1$	$T=2086$ $P=0.5$	$T=2167.5$ $P=0.18$	
<i>Liolaemus lineomaculatus</i>							
Esquel	25.8 \pm 0.7 (52)	34.5 \pm 0.4 (60)	32.1 \pm 0.8 (60)	37.8 \pm 0.2 (60)	6.9 \pm 0.8 (53)	12.6	0.45
Perito Moreno	26.1 \pm 0.4 (90)	32 \pm 0.5 (73)	28.9 \pm 0.7 (73)	35.9 \pm 0.4 (73)	4.5 \pm 0.4 (73)	7.7	0.41
Comparisons	$F_{1,142}=3.98$ $P=0.05$	$F_{1,133}=6.34$ $P < 0.001$	$T=4888$ $P < 0.001$	$T=5151$ $P < 0.001$	$T=3755.5$ $P=0.06$	$T=4748.5$ $P < 0.001$	

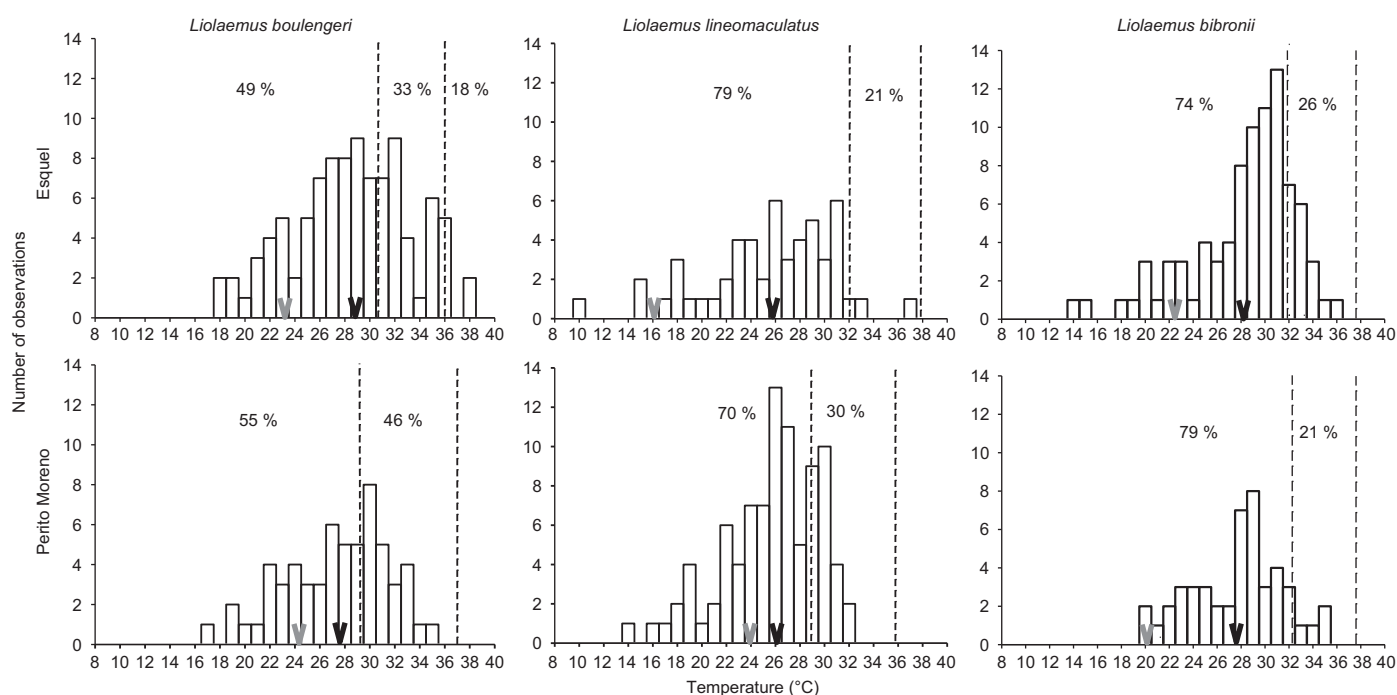


Fig. 3. Distribution of body temperatures (T_b) at capture of *Liolaemus boulengeri*, *L. lineomaculatus* and *L. bibronii* (bars) in Esquel and Perito Moreno. The black arrows show the mean T_b , grey arrows the mean T_{pref} and the dashed lines indicate the lower and upper set-points of the preferred body temperature (T_{set}).

4. Discussion

The T_b s *L. boulengeri* ($T_b=28^\circ\text{C}$) and *L. lineomaculatus* (26°C) experience in their natural environments are the lowest for oviparous *Liolaemus* species ($29\text{--}37^\circ\text{C}$, Medina et al., 2009), but similar to the southernmost viviparous lizards in the world, *Liolaemus sarmientoi* and *L. magellanicus* (26 and $23\text{--}27^\circ\text{C}$, respectively; Jaksic and Schwenk, 1983; Iburgüengoytia et al., 2010). Low T_b s have also been found in the viviparous genus *Phymaturus* (*Liolaemidae*) from cold environments at high latitude in the Patagonian steppe ($39\text{--}41^\circ\text{S}$; *P. tenebrosus*: 29 ; *P. zapalensis*: 26.4°C ; Iburgüengoytia, 2005; Iburgüengoytia et al., 2008; Cruz et al., 2009) or from high altitude in northern lands ($30\text{--}37^\circ\text{S}$; $2000\text{--}4200$ m asl) such as *P. dorsimaculatus* (22.5°C ; Cruz et al., 2009) and *P. punae* (29.4°C ; Iburgüengoytia et al., 2008).

In contrast, *Liolaemus boulengeri*, *L. lineomaculatus* and *L. bibronii* preferred higher temperatures in the laboratory (approximately, 34°C), than those they can achieve in their environments, similar to the rest of the *liolaemids* ($34\text{--}36^\circ\text{C}$, Labra, 1998; $31\text{--}34$ and $30\text{--}37^\circ\text{C}$, Medina et al., 2009). The

difference between T_b and T_{pref} is especially steep in *L. magellanicus* and *L. sarmientoi*, which are far from achieving T_{pref} and the optimal T_b to reach their maximum performance (T_o) in their natural environment (Fernández et al., 2011).

The similar T_b s within species populations of *Liolaemus boulengeri*, *L. lineomaculatus* and *L. bibronii* (Esquel at 1400 m asl and Perito Moreno) show they adjust thermoregulation to compensate for the differences in the availability of thermal micro-environments (T_e) and in wind intensity using different heat sources and behaviours. *Liolaemus boulengeri* and *L. lineomaculatus*, as well as *L. bibronii*, even when they are syntopic, choose different micro-environments that offer diverse challenges impelling lizards to find different paths for thermoregulation. The higher T_b than T_a , T_{ssh} and T_e in both populations of *L. boulengeri* and *L. lineomaculatus* showed that lizards loose heat by convection and move to thermoregulate from sunny to shade substrates. But, the differences in the relationship between T_b with T_{ss} found in *L. boulengeri* and *L. lineomaculatus* indicate that in Esquel and Perito Moreno, *L. boulengeri* choose micro-environments that offer higher opportunities to elevate their T_b by substrate. In contrast,

L. lineomaculatus was found especially in cooler micro-environments, at a higher elevation site, in Esquel. The strong relationship of T_b with substrate temperatures in the sun in *L. lineomaculatus*, and not with the illuminance, suggests that the main heat sources to raise T_b are the sunny substrate patches rather than radiation, and thus this species elevates T_b mainly by thigmothermy. In contrast, T_b s lower than the sunny substrate temperature, in Esquel and Perito Moreno *L. boulengeri* populations, are characteristic of heliothermy. The congeners from higher latitudes, such as *L. magellanicus* and *L. sarmientoi* (50–52°S), show a clear opportunistic thermoregulatory behaviour, being heliothermic when air temperature is cold during the morning and turning to thigmothermy in the warmest hours of the day, early afternoon (Ibargüengoytía et al., 2010). In addition, the oviparous *L. boulengeri* is able to thermoregulate more effectively than the syntopic *L. lineomaculatus* and *L. bibronii*. The combination of d_b and d_e indices showed an active selection of suitable thermal micro-environments, confirming that *L. boulengeri* can thermoregulate more precisely and select the available thermal resources with higher effectiveness ($E=0.55$) than the viviparous *L. lineomaculatus* ($E=0.43$) or the oviparous *L. bibronii* ($E=0.44$; Medina et al., 2009). Nevertheless, there is a complex interplay among ecological and physiological traits determining the distribution and survivorship of the species, since *L. lineomaculatus* has the southernmost distribution of these three species.

Thermal relationships between body temperature and environment conditions set upper distributional limits on oviparous species, which cannot successfully reproduce at high elevations, where only viviparous lizards are found (Carothers et al., 1998). Although *L. boulengeri* lives in syntopy with *L. lineomaculatus* and the oviparous *L. bibronii* in some parts of their distribution, *L. lineomaculatus* shows the most austral distribution (from 37 to 53°S) compared with both, *L. boulengeri* and *L. bibronii* (from 31 to 50°S). The main advantage of viviparity is the retention of the eggs within the uterus until embryonic development is complete. Gravid females can thermoregulate to keep embryos warmer and maintain a more stable developmental temperature than they would be in a nest exposed to environmental conditions (Andrews, 2000). This appears to have evolved frequently among squamate reptiles in cool environments at high latitudes and elevations (Shine, 1985; Shine and Bull, 1979; Tinkle and Gibbons, 1977). Instead, oviparous species in cold climates restrict the laying season to early summer (Shine, 1985; Medina and Ibargüengoytía, 2010), allowing hatchlings to grow and store sufficient energy prior to the onset of winter (Olsson and Shine, 1997). Nevertheless, this explanation is still incomplete since *L. boulengeri*, *L. bibronii* and *L. lineomaculatus* show almost identical timing in the reproductive cycles and the viviparous *L. lineomaculatus* shares the same high latitudes with other oviparous species such as *Diplolaemus darwini* (45–52°S), and the nocturnal and oviparous *Homonota darwini* (34–52°S; Cei, 1986; Scolaro, 2005).

Acknowledgments

We wish to express our gratitude to E. Forti and V. Forti for their help in the field. This work was partially supported by Universidad Nacional del Comahue (04B129), CONICET (PIP 100271) and NSF Grant IOS-1022031.

References

Andrews, R.M., 2000. Evolution of viviparity in squamate reptiles (*Sceloporus* spp.): a variant of the cold-climate model. *J. Zool.* 250, 243–253.

- Angilletta Jr., M.J., 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis, first edition Oxford University Press (p. 304).
- Avery, R.A., 1978. Activity patterns, thermoregulation and food consumption in two sympatric lizard species (*Podarcis muralis* and *P. sicula*) from central Italy. *J. Anim. Ecol.* 47, 143–158.
- Bakken, G.S., 1992. Measurement and application of operative and standard operative temperatures in ecology. *Am. Zool.* 32, 194–216.
- Bauwens, D., Hertz, P.E., Castilla, A.M., 1996. Thermoregulation in a lacertid lizard: the relative contributions of distinct behavioral mechanisms. *Ecology* 77, 1818–1830.
- Beaupre, S.J., 1995. Effects of geographically variable thermal environment on bioenergetics of mottled rock rattlesnakes. *Ecology* 76, 1655–1665.
- Carothers, J.H., Fox, S., Marquet, P., Jaksic, F., 1998. Thermal characteristics of ten Andean lizards of the genus *Liolaemus* in Central Chile. *Rev. Chil. Hist. Nat.* 70, 297–309.
- Cei, J., 1986. Reptiles del Centro-oeste y Sur de la Argentina. Herpetofauna de las Zonas Áridas y Semiáridas first edition Museo Regionale di Scienze Naturali, Monografia IV, Torino, Italy.
- Cruz, F.B., Belver, L., Acosta, J.C., Villavicencio, H.J., Blanco, G., Cánovas, M.G., 2009. Thermal biology of *Phymaturus* lizards: evolutionary constraints or lack of environmental variation? *Zoology* 112, 425–432.
- Dorcas, M.E., Peterson, C.R., Flint, M.E.T., 1997. The thermal biology of digestion in rubber boas (*Charina bottae*): physiology, behaviour, and environmental constraints. *Physiol. Zool.* 70, 292–300.
- Dzialowski, E.M., 2005. Use of operative temperature and standard operative temperature models in thermal biology. *J. Therm. Biol.* 30, 317–334.
- Fernández, J.B., Smith Jr., J., Scolaro, A., Ibargüengoytía, N.R., 2011. Performance and thermal sensitivity of the southernmost lizards in the world, *Liolaemus sarmientoi* and *Liolaemus magellanicus*. *J. Therm. Biol.* 36, 15–22.
- Gutiérrez, J.A., Krenz, J.D., Ibargüengoytía, N.R., 2010. Effect of altitude on thermal responses of *Liolaemus pictus argentinus* in Argentina. *J. Therm. Biol.* 35, 332–337.
- Halloy, M., Laurent, R., 1988. Notes eco-ethologiques sur *Liolaemus huacahuasicus* Laurent (Iguanidae) du Nord-Ouest argentin. *Rev. Fr. Aquariol.* 14, 137–144.
- Hertz, P.E., Huey, R.B., 1981. Compensation for altitudinal changes in the thermal environment by some *Anolis* lizards on Hispaniola. *Ecology* 62, 515–521.
- Hertz, P.E., 1992. Temperature regulation in Puerto Rican *Anolis* lizards: a field test using null hypotheses. *Ecology* 73, 1405–1417.
- Hertz, P.E., Huey, R.B., Stevenson, R.D., 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *Am. Nat.* 142, 796–818.
- Hill, R.W., 1980. In: Harper, Row (Ed.), *Fisiología Animal Comparada, un enfoque ambiental*. Reverté, Barcelona, España.
- Huey, R.B., Slatkin, M., 1976. Costs and benefits of lizards thermoregulation. *Q. Rev. Biol.* 51, 363–384.
- Ibargüengoytía, N.R., 2005. Field, selected body temperature and thermal tolerance of the syntopic lizards *Phymaturus patagonicus* and *Liolaemus elongatus* (Iguania: Tropiduridae). *J. Arid Environ.* 62, 435–448.
- Ibargüengoytía, N.R., Acosta, J.C., Boretto, J.M., Villavicencio, H.J., Marinero, J.A., Krenz, J.D., 2008. Field thermal biology in *Phymaturus* lizards: comparisons from the Andes to the Patagonian steppe in Argentina. *J. Arid Environ.* 72, 1620–1630.
- Ibargüengoytía, N.R., Medina, M., Fernández, J.B., Gutiérrez, J.A., Tappari, F., Scolaro, A., 2010. Thermal biology of the southernmost lizards in the world: *Liolaemus sarmientoi* and *Liolaemus magellanicus* from Patagonia, Argentina. *J. Therm. Biol.* 35, 21–27.
- Jaksic, F.M., Schwenk, K., 1983. Natural history observations on *Liolaemus magellanicus*, the southernmost lizard in the world. *Herpetology* 4, 457–461.
- Labra, A., 1998. Selected body temperature of seven species of Chilean *Liolaemus* lizards. *Rev. Chil. Hist. Nat.* 71, 349–358.
- Marquet, P.A., Ortiz, J.C., Bozinovic, F., Jaksic, F.M., 1989. Ecological aspects of thermoregulation at high altitudes: the case of Andean *Liolaemus* lizards in northern Chile. *Oecologia* 81, 16–20.
- Medina, M., Gutiérrez, J., Scolaro, A., Ibargüengoytía, N.R., 2009. Thermal responses to environmental constraints in two populations of the oviparous lizard *Liolaemus bibronii* in Patagonia, Argentina. *J. Therm. Biol.* 34, 32–40.
- Medina, M., Ibargüengoytía, N.R., 2010. How do viviparous and oviparous lizards reproduce in Patagonia? A comparative study of three species of *Liolaemus*. *J. Arid Environ.* 74, 1024–1032.
- Morando, M., Avila, L.J., Turner, C.R., Sites Jr., J.W., 2007. Molecular evidence for a species complex in the patagonian lizard *Liolaemus bibronii* and phylogeography of the closely related *Liolaemus gracilis* (Squamata: Liolaemini). *Mol. Phylogenet. Evol.* 43, 952–973.
- Niewiarowski, P.H., 2001. Energy budget, growth rates, and thermal constraints: toward an integrative approach to the study of life history variation. *Am. Nat.* 157, 421–433.
- Norusis, M.J., 1986. *Advanced Statistics SPSS/PC+ for the IBM PC/XT/AT*. SPSS Inc., Chicago.
- Olsson, M., Shine, R., 1997. The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why earlier clutches are better. *J. Evol. Biol.* 10, 369–381.
- Paruelo, J.M., Jobbagy, E.G., Sala, O.E., Lauenroth, W.K., Burke, I.C., 1998. Functional and structural convergence of temperate grassland and shrub land ecosystems. *Ecol. Appl.* 8, 194–206.
- Pearson, O.P., 1954. Habits of the lizard *Liolaemus multififormis multififormis* at high altitudes in southern Peru. *Copeia* 2, 111–116.

- Pincheira-Donoso, D., Scolaro, A., Sura, P., 2008. A monographic catalogue on the systematics and phylogeny of the South American iguanian lizard family Liolaemidae (Squamata, Iguania). *Zootaxa* 1800, 1–85.
- Pough, F., Gans, C., 1982. The vocabulary of reptilian thermoregulation. In: Gans, C. (Ed.), *Biology of Reptilia*, vol. 12. , Wiley, New York, pp. 17–23.
- Scolaro, A., 2005. *Reptiles Patagónicos: Sur. Guía de Campo*. Universidad Nacional de la Patagonia, Trelew, Argentina.
- Sears, M.W., 2005. Geographic variation in the life history of the sagebrush lizard: the role of thermal constraints on activity. *Oecologia* 143, 25–36.
- Shine, R., Bull, J.J., 1979. The evolution of live-bearing in lizards and snakes. *Am. Nat.* 113, 905–923.
- Shine, R., 1985. The reproductive biology of Australian reptiles: A search for general patterns. In: Gordon, G., Shine, R., Ehmann, H. (Eds.), *Biology of Australasian Frogs and Reptiles*, Royal Zoological Society of New South Wales, pp. 297–303.
- Sinervo, B., Mendez-De La Cruz, F., Miles, D.B., Heulin, B., Bastians, E., Villagran-Santa Cruz, M., Lara-Resendiz, R., Martínez-Méndez, M., Calderon-Espinosa, M., Mesa-Lázaro, R., Gadsden, H., Avila, L., Morando, M., De La Riva, I., Victoriano, P., Duarte Rocha, C., Ibarquengoytia, N.R., Puntriano, C., Massot, M., Lepetz, V., Okansen, T., Chapple, D., Bauer, A., Branch, W., Clobert, J., Sites Jr., J., 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328, 894–899.
- Sokal, R.R., Rohlf, F., 1969. *Biometry. The Principles and Practice of Statistics in Biological Research*. W.H. Freeman and Co., San Francisco, CA.
- Tinkle, D.W., Gibbons, J.W., 1977. The distribution and evolution of viviparity in reptiles, vol. 154. *Miscellaneous Publications Museum of Zoology, University of Michigan* (pp. 1–54).
- Zug, G.R., Vitt, L.G., Caldwell, G.P., 2001. *Herpetology: An Introductory Biology of Amphibians & Reptiles*, 2nd edition Academy Press (p. 630).