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Effect of altitude on thermal responses of *Liolaemus pictus argentinus* in Argentina

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**Abstract**

Reptiles that live in cooler environments hibernate longer and, when active, limit daily activity times, allocate more time and energy toward thermoregulation, and consequently experience life-history constraints such as reduced fecundity and supra-annual reproductive cycles. This pattern becomes more extreme with increasing latitude and altitude. We compared the thermal biology of two populations of *Liolaemus pictus argentinus* living at two altitudes (771 and ~1700 m asl). Environmental, microenvironmental, and operative temperatures were studied in order to describe the capture sites, sources of heat, and availability of microenvironments appropriate for thermoregulation. The body temperatures of *L. p. argentinus* at capture (*T*<sub>c</sub>) and the preferred temperatures in the laboratory (*T*<sub>p</sub>) were recorded and integrated with operative temperatures to calculate the effectiveness of thermoregulation. The high-altitude population was found to have a lower mean *T*<sub>c</sub> (29 °C compared with 33 °C), while the *T*<sub>p</sub> values for both populations were similar (36.7 °C). The analysis of operative temperatures and *T*<sub>p</sub> in relation to *T*<sub>c</sub> showed that *L. p. argentinus* behaves as a moderate thermoregulator at high altitude and as a poor thermoregulator at the low-altitude site probably due in part to the avoidance of predation risk.

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and pristidactylus volcanensis in Chile that inhabit the closed-canopy Nothofagus forest versus open-canopy forests, respectively (Labra and Rosenmann, 1992).

Liolaemus pictus argentinus (Liolaemidae) is a viviparous and insectivorous species with a wide distribution in the Patagonian Andes of Neuquén, Río Negro, and Chubut provinces of Argentina (39°43′ S and 520–1600 m asl (Donoso-Barros, 1966; Cej, 1986; Scolaro, 2005)). Previous studies of L. p. argentinus (Ibargüengoytia and Cussac, 2002), Liolaemus elongates, and Phymaturus tenebrosus (Ibargüengoytia, 2005; Ibargüengoytia et al., 2008) suggest that environments characterized by low temperatures throughout the year and short activity seasons limit the opportunities for thermoregulation and in turn influence several life-history traits. These species are predominantly heliothermal and at low altitude (Ibargüengoytia and Cussac, 2002) a mean \( T_b \) of 33.2 °C was observed, which is similar to that of other liolaemids (32.5 °C, \( N = 45 \) lizards; Medina et al., 2009). Herein we report differences in thermal physiology in L. p. argentinus between high and low altitude populations in close proximity.

2. Materials and methods

2.1. Study areas and field methods

The two field sites in northwestern Patagonia, Argentina, are Cerro Chullhuanco (41 15°57′9″ S and 71°17′57′ W; 1615–1769 m asl) and Melipal Beach on lake Nahuel Huapi (41°47′41.53′′ S and 71°20′44.87′′ W, 771 m asl), both near the city of San Carlos de Bariloche in Río Negro Province. Lizards (\( N = 30 \)) were captured by loop or hand at high altitude in December 2005, in January, April, and December 2006, and in February 2007. At low altitude, 33 lizards were captured in February and March of 2006 and 2007. All captures were authorized by the Wild Life Service of the Province of Río Negro and Nahuel Huapi National Park.

The microenvironment at each capture site was described by measuring the following variables: substratum temperature (\( T_s \), TESTP-K03 substrate probe) and air temperature 1 cm above the ground (\( T_a \), TESTP-K02 gas probe) recorded with thermocouples connected to a TES 1302 thermometer (TES Electrical Electronic Corp., Taipei, Taiwan, ±0.03 °C), wind velocity (Turbometer, ±0.1 m/s), humidity (Micro-meteorological Station, Lutron LM-8000), and luminescence (Luximeter Extech model 401025, ±1 lux).

Body temperatures \( (T_b) \) were measured in the cloaca using a thermocouple (TES TP-K01; 1.62 mm diameter). Lizards were handled by the head and the measurements were done within 20 s after capture in order to minimize heat transfer to the animal. A data logger (HOBO) was installed at each field site to record environmental temperatures hourly for 1 yr in order to estimate the mean environmental temperature. In addition, 20 copper models were installed in different microenvironments at each site—the size of the model was 63 \times 10 \times 3 \, mm³ and they were hollow copper pipes painted black and a small hole allowed the insertion of a thermocouple. Temperatures of the models were tested simultaneously against the body temperatures \( (T_b) \) of live L. p. argentinus under a heat source in laboratory. Temperatures of the model and the live individual showed a tight correlation (\( r^2 = 0.90, P < 0.0001, N = 50 \); Ibargüengoytia et al., 2009), confirming that the models acquired temperatures similar to that of a non-thermoregulating lizard. The temperatures were recorded 3 or 4 times daily for each model to estimate the mean operative temperatures \( (T_{ot}) \).

2.2. Estimation of preferred body temperatures

Body temperature preference experiments were conducted the day after capture. Lizards were placed individually in open-top terraria (200 \times 45 \times 18 \, cm³) each with a sand floor and a thermal gradient produced by a line of four infra-red lamps overhead (one 250 W, two 150 W, and one 100 W). The lamps were adjusted to different heights to make a linear substratum gradient from 15–69 °C. Lizard body temperatures were measured every 10 min for 5 h using ultra thin (1 mm) catheter thermocouples located approximately 10 mm inside the cloaca and fastened to the base of the lizard’s tail to keep the thermocouple in position during the experiment (TES 1302 thermometer, TES Electrical Electronic Corp., Taipei, Taiwan, ±0.03 °C). All measurements were taken so as to minimize interference with their normal activities. The duration of the experiments corresponded to previous trials that measured the amount of time required for Liolaemus biruboni (Medina et al., 2009), Liolaemus pictus (Gutiérrez, 2009), and several other liolaemids (Liolaemus lineomaculatus, Liolaemus boulengeri, L. elongatus, and Liolaemus fitzingeri; Ibargüengoytia, unpublished data) to reach their preferred temperature asymptote.

We estimated the mean and range of the preferred body temperature \( (T_p) \) for each individual. The set-point range \( (T_{set}) \), considered as the temperatures within the interquartile range of the observations, was also noted because earlier studies show neurophysiological evidence that ectotherms regulate between upper and lower set-point temperatures rather than around a single \( T_b \) (Barber and Crawford, 1977; Firth and Turner, 1982). The interquartile range represents the natural settings caused by the hypothalamic thermostat in lizards and fishes (Barber and Crawford, 1977; Firth and Turner, 1982). In order to measure the average extent to which L. p. argentinus experienced \( T_p \) values outside the set-point range, the sum of absolute values of the deviations of \( T_b \) from \( T_{set} \) of each individual was calculated (individual deviation—\( d_b \)). The \( d_b \) values obtained for each lizard were used to estimate the mean and range of \( T_p \), \( T_{set} \), and mean \( d_b \) of each population.

The index of mean thermal quality of the habitat \( (d_h) \) was calculated as the deviation of \( T_b \) from the mean \( T_{set} \) for each population. The existence of active selection of the microhabitats and the effectiveness of thermoregulation was obtained as \( E = 1 - (d_h/d_b) \); this formula integrates the average degree to which the lizards experienced \( T_p \) values outside the set-point range \( (d_b) \) and the corresponding \( d_b \). 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; Medina et al., 2009).

2.3. Statistical analyses

We used the statistical software programs Sigma Stat 3.5®, SPSS® 11.0, and 10.0® Sigma Plot for statistical analysis. The dependence between variables was analyzed by simple or multiple stepwise regressions. The differences between sample means were analyzed using paired, when observations were related, or unpaired t-tests. For more than two tests, we used a one-way analysis of variance for repeated measures. 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 or Kruskal–Wallis tests for comparison of means of two independent samples. The significance level used
for all statistical tests was 5% (Sokal and Rohlf, 1969; Norusis, 1986).

3. Results

3.1. Body sizes

Thirty-three animals were captured at each site. At both capture sites all the specimens were captured outside shelters, while feeding, basking, or during activities related to reproduction from 11:00 to 18:00 h. There was no difference between sites in either body mass (t-test, t_{53} = 1.83; P > 0.07) or SVL (Mann–Whitney, T_{SVL} = 1022.00, N = 66, P > 0.29).

3.2. Environmental and body temperatures

Mean monthly temperatures differed between sites year-round except during December, January, March, and April (Fig. 1). The environmental temperatures at high altitude stayed below 0°C from June to October while at low altitude the lower values were observed from June to September but monthly means were never below 0°C. In December and January, the low altitude site was cooler (Fig. 1).

The mean \( T_b \) was significantly warmer than \( T_s \) and \( T_a \) in both populations (paired t-tests, high altitude: \( t_{T_b,T_s} = 2.41, P < 0.001; \) low altitude: \( t_{T_b,T_s} = 5.85, P < 0.001; \) Fig. 2 and Table 1). Mean \( T_s \) was significantly cooler at high altitude (\( t_{61} = 3.16, P < 0.002; \) Table 2).

3.3. Operative temperatures (\( T_e \))

At high altitude, \( T_e \) were significantly lower than \( T_b \) (Mann–Whitney, \( T_{69} = 1275.0, P < 0.002, \) median \( T_e = 24.9 °C, \) median \( T_b = 28.8 °C \). At low altitude, \( T_e \) did not differ significantly from

**Fig. 1.** Mean monthly temperature at the high altitude site (circles) and low altitude site (squares). Regression and confidence intervals (95%) were drawn considering the best-fit curve generated by the program Table Curve 2D.

**Fig. 2.** Relationship between body temperature and substratum temperatures (\( T_s \)) and air temperatures (\( T_a \)). Dashed lines indicate \( Y = X \) relationships. Curves indicate 95% confidence intervals. Solid lines are the temperature set-points.

Table 1
Physical environmental characteristics of high and low altitude sites. Humidity (H%), luminosity (Luxes), wind velocities (m/sec), substrate temperature (Ts, °C) and air temperature (Ta, °C). Values shown are mean, ± standard error, (sample size), and range.

<table>
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<th>Altitude</th>
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<th>Luminosity</th>
<th>Environmental characteristics</th>
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<tr>
<td></td>
<td></td>
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<td>Wind</td>
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<tr>
<td>High</td>
<td>27.36 ± 1.53 (21)</td>
<td>1131.5 ± 103.6 (30)</td>
<td>226–2200</td>
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<tr>
<td>Low</td>
<td>29.07 ± 1.41 (33)</td>
<td>1232.9 ± 98.6 (33)</td>
<td>230–2900</td>
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Table 2
Thermal statistics for each site. Values shown are mean, ± standard error, or median and (sample size). Ranges are also shown for body temperature (Tb) and operative temperature (Te). Other measures shown are preferred body temperature (Tp), index of the average thermal quality of a habitat from an organism’s perspective (de), and individual deviation of Tb from Tset (db). All temperatures are in °C.

<table>
<thead>
<tr>
<th>Altitude</th>
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<th>Tp</th>
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<td>High</td>
<td>28.9 ± 0.8 (30)</td>
<td>(22.7–37.0)</td>
<td>24.9 (44) (20.6–37.9)</td>
<td>36.2 ± 0.4 (27)</td>
<td>9.47 (40) (5.6 ± 0.8 (27)</td>
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<tr>
<td>Low</td>
<td>32.6 ± 0.9 (33)</td>
<td>(22.3–43.5)</td>
<td>31.3 (77) (17.7–59.5)</td>
<td>35.6 ± 0.5 (30)</td>
<td>5.20 (77) (6.5 ± 1.0 (30)</td>
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Fig. 3. Frequency of body temperature (Tb, bars) of lizards from high (panel A) and low altitude (panel B) sites. The arrows indicate mean Tb and Tp and the vertical lines indicate the lower and upper set-point of the Tp (dashed lines) and the median of operative temperature (solid line, Te).
3.4. Preferred temperatures in the laboratory (T_p)

At high altitude, T_p ranging from 31 to 39.1 °C, was significantly larger than T_b (paired t-test, t_{29}=11.15, P < 0.0001). At low altitude, the range of T_p, varied from 32.6 to 39.3 °C and was significantly larger than T_b (paired t-test, t_{29}=−3.73, P < 0.001). The T_p values were not different between sites (Mann–Whitney, T_{157}=8.25, P = 0.51), and did not differ between localities within groups of males, females, or juveniles (Kruskal–Wallis, H_{2;57}=1.47, P > 0.48). High altitude set points were 34.3 ± 0.7 °C, and 38.2 ± 0.3 °C while the low altitude set points were 34.6 ± 0.2 °C and 38.0 ± 0.3 °C (Fig. 3; Table 2).

3.5. Individual deviation values (d_v) index of mean thermal quality of the habitat (d_h), and effectiveness of thermoregulation (E)

The individual deviation values (d_v) were 5.6 ± 0.8 (N = 27) at high altitude and 6.5 ± 1.0 (N = 30) at low altitude. The individual deviation values (d_v) were not significantly different between sites (t-Student, t_{55}=1.83, P > 0.07). Nevertheless, 81% of the T_b values at high altitude were lower than the lower set-point, while 73% of the T_b values at low altitude were greater than the upper set-point.

The index of mean thermal quality of the habitat (d_h) was 9.5 (N = 40) in the high altitude site and 5.2 (N = 77) in the low altitude site. Most of the T_b values at high altitude (97.5%) were lower than the lower set-point. But, this was true only for 62.4% of the T_b values at low altitude. In addition, some low altitude values (24.6%) were greater than the upper set-point. The values of d_v were significantly greater at high altitude (Mann–Whitney, T_{117}=2850, P < 0.005; median high = 9.5 °C and median low = 5.2 °C). The E values were 0.404 and −0.242 at high and low altitude, respectively.

4. Discussion

Monthly mean air temperatures and operative temperatures at the high-elevation site, in the Nothofagus forest near the summit of Challhuaco Mountain, were lower than those at the low-elevation site (Fig. 1). Although from April to August the temperature is low at both sites, at high altitude the lower temperatures last until October, leaving the lizards inactive in their burrows under the snow, while at the beach the mean temperature in September was 4 °C and lizards were active in the warmest part of the day. Nevertheless, the mean temperatures, from mid-November to mid-January were warmer at high altitude, possibly due to greater radiation there and due to the buffering effect of water at the beach (Koeppen, 1948).

Lizards take advantage of thermal resources through thermoregulatory behavior (Randall et al., 1997) and differences in behaviors and activity periods have been observed among latitudinal (Medina et al., 2009) and altitudinal gradients (Hertz et al., 1983). However, optimal thermoregulation may trade off with costs in feeding rate, reproductive activity, and predation risk. We observed lower activity temperatures at high elevation (29 °C compared with 33 °C), which may be explained by differences in ambient temperature between the two sites. A similar pattern was observed in Agama savignyi and Stellio stellio from Israel (Hertz et al., 1983) and in the genus Sceloporus, whose mean body temperatures in tropical latitudes decrease with altitude from 35 to 31 °C (Andrews, 1998).

Our analysis of microenvironmental temperatures and T_b shows that this species gains heat mainly through solar radiation and loses heat passively by conduction and convection, evidence of their heliothermic character, and this corroborates the results obtained by Ibariguengoytia and Cussac (2002; Fig. 2). The lower body temperatures we observed at high altitude were likely caused by shady conditions under the closed forest canopy. On the other hand, at the low altitude beach, although less protected from the wind, lizards usually had sun-lit conditions and rocky microenvironments and thus a greater thermal inertia. These conditions apparently allowed lizards to maintain higher body temperatures and to lose less heat by conduction. This difference could also be explained by differences in ambient temperature between the two sites.

The T_b values at the high altitude site were similar to that of other Patagonian lizards from similar latitudes in the steppe environment such as L. bibronii (28 °C; Medina et al., 2009) and L. elongatus (29.8 °C; Nuoriš, 1986). In contrast the body temperatures of lizards at the low-latitude site were similar to that of L. elongatus and L. p. argentinus (33.2 °C) obtained on the shore of Moreno Lake (Ibariguengoytia and Cussac, 2002) and with those of Liolaemus wiegmanni (32–36 °C; Martori et al., 1998) and Liolaemus koslowskii (34.8 °C; Martori et al., 2002), which inhabit warmer environments at lower latitudes in Rio Cuarto in the Province of Cordoba, and on the plains of Monte de Anillaco in La Rioja, respectively.

The range of body temperatures during activity in the field can vary according to body mass and size and shape of the body (Christian, 1998) and also according to reproductive conditions (Smith and Ballinger, 1994). Many studies of lizard thermoregulation show T_b values outside of the set-points temperatures in their natural environment due to different factors such as the presence of predators, low availability of thermal microenvironments, or restrictions imposed by sociality or stage of reproduction (Hertz et al., 1983; Autumn and De Nardo, 1995). Thermoregulation in many occasions may be effective, but lizards that move between sun and shade use energy in locomotion, which otherwise could be used for reproduction, growth, or other functions (Huey and Slatkin, 1976). Consequently, the most adaptive thermoregulatory behavior may not result in the best match between body temperature and preferred temperature. Instead, for example, passive thermoregulation in lizards that inhabit cold climates or shady forests can be from time to time more beneficial than an active or very precise thermoregulation (Huey and Slatkin, 1976). We measured preferred temperatures that were significantly warmer than T_b values at the time of capture, probably due to a scarcity of thermal microenvironments that could be used to obtain high temperatures in the wild. Nevertheless, T_p values were not different between the two populations (36 °C). The preferred temperatures we observed were similar to a report for Liolaemus (34–36 °C; Labra, 1998) and others (Medina et al., 2009; 30–37 °C), suggesting conservation of this character within the species and the genus (Labra, 1998). The latter study also found strong evidence for a phylogenetic effect on T_p, while T_b seems to be the result of adaptation to the local temperature and climate (Labra, 1998; Labra et al., 2009).

The availability of favorable microenvironments can be indicated by T_e and by the difference between operative temperatures and the set-point of preferred temperatures determined in the laboratory (d_v). Operative temperatures were lower (median T_e=24.9 °C) and the d_v was greater (median d_v=9.5 °C) at high altitude compared with low altitude (median T_e=31.3 °C, median d_v=5.2 °C), indicating lower availability of thermal environments on the mountain. These high d_v values were similar to those found for the steppe lizard L. bibronii (d_v=8.4–10.9 °C; Medina et al., 2009) but much greater.
than those found for Anolis populations in tropical climates ($d_b$=0.1–9.2 °C; Sinervo et al., 2010). Podarcis hispanica atrata ($d_b$=6.3 °C; Bauwens et al., 1996), and for the genus Varanus ($d_b$=2–8 °C; Christian and Weavers, 1996), likely the result of the rigors of living in the cold microenvironments in Patagonia.

Likewise, $d_b$ values indicate the difference between field body temperatures and those preferred in the laboratory. The lower and upper set-points in both localities were similar and no significant differences were found in $d_b$. Nevertheless, because body temperatures were mostly below the set-point range at high altitude but above the set-point range at low altitude (Fig. 3), we conclude that the forested mountain environment provides little opportunity for thermoregulation (high $d_b$). In contrast, the $T_b$ of lizards at the beach often exceeded their preference, a result probably not due to low availability of environments but due to other factors, likely biotic. Possibly, their prey is located in warmer microenvironments, forcing the lizards to expose themselves to higher temperatures. Additionally, they may be evading potential predators (including humans, which may be perceived as predators), thereby placing themselves in microenvironments that are thermally suboptimal (Fig. 3).

Efficiency in the regulation of temperature, indicated by $E$, characterizes L. p. argentinus as a moderate thermoregulator at high altitude ($E=0.4$) and more as a thermoconformer at low altitude ($E=0.24$). In the former case, it appears that a lack of suitable thermal microenvironments prevents the attainment of $T_F$ (Ibargüengoytía et al., 2009) in the latter case, a negative $E$-value occurs when lizards have favorable thermal microenvironments available but other functions, such as interactions with predators or prey, constrain their use of them (Sinervo et al., 2010). For example, in the radiotelemetry study of thermal biology on Varanus spp. from Australia, one species exhibited $E=-0.2$ during the dry season because lizards were mostly in their refuges (Christian and Weavers, 1996). Behavioral field studies would allow further elucidation of the causes of the differences in thermoregulation in comparative studies like ours.

Acknowledgements

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