High-mountain altitudinal gradient influences thermal ecology of the Mesquite lizard Sceloporus grammicus.

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High-mountain altitudinal gradient influences thermal ecology of the Mesquite lizard *Sceloporus grammicus*.

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High-mountain altitudinal gradient influences thermal ecology of the Mesquite lizard *Sceloporus grammicus*.

**Abstract**

The thermal requirements of ectotherms may vary among species due to adaptation to different thermal environments. Nevertheless, some of these requirements are evolutionarily conserved, leading organisms to compensate behaviorally for harsh environmental conditions. High-mountain systems provide temperature gradients that allow for studies of evolutionary and plastic variation in thermal ecology under natural conditions. We evaluated the thermoregulation strategies of *Sceloporus grammicus* Wiegmann, 1828 at three points (2600, 3100, and 4150 m asl) along an altitudinal gradient. We found that the thermal quality of the site and the body temperatures of lizards are influenced by altitude and decrease with increasing elevation. However, lizards from the three different elevations have similar thermal requirements. High-altitude lizards have lower thermal accuracy and efficiency indexes in comparison to the lower sites, due to the low thermal quality of their environment. Nevertheless, they are efficient in thermoregulation, increasing their body temperature above the ambient temperature. We found that pregnant females from all three elevations had similar preferred body temperatures. In comparison with non-pregnant females and males, they exhibited lower preferred temperatures and more accurate thermoregulation. The wide altitudinal distribution of *S. grammicus* is thus not caused by variable thermal requirements. Instead,
these lizards’ wide repertoire of physiological and behavioral strategies allows this species to successfully inhabit contrasting environments.

Key words: thermal efficiency, thermal quality, Mesquite lizard, *Sceloporus grammicus.*

**Volcano.**

**Introduction**

Temperature is one of the most important abiotic factors for ectotherms because it affects all biological functions, having a direct impact on fitness (Hertz et al. 1993; Blouin-Demers and Weatherhead 2001; Lourdais et al. 2004; Andrews and Schwarzkopf 2012). Hence, the thermal requirements of ectotherms (e.g. lizards) are expected to vary according to the thermal characteristics of their habitats (Van Damme et al. 1989; Webber and Bryson 2015). Natural selection can favor a rapid population response to variable thermal environments, producing local adaptation (Logan et al. 2018) or acclimation (i.e. phenotypic plasticity; West-Eberhard 2003; Paranjpe et al. 2013).

In general, temperature decreases as latitude and altitude increase, which makes habitats at high latitudes and altitudes (e.g. high-mountain systems) particularly hostile for ectotherms (Gvoždík 2002; Angilleta 2009; Aguado and Braña 2014). In particular for lizards, these low temperature habitats are often also low thermal quality environments (*de*), calculated as the extent to which the habitat allows an ectotherm to achieve their preferred body temperature range (Hertz et al. 1993). Based on the cost-benefit model proposed by Huey and Slatkin (1976), the lizards inhabiting colder environments also will exhibit lower efficiency of thermoregulation (*E*), which is the capacity of an animal to
thermoregulate with the available resources through physiological and behavioral processes (Hertz et al. 1993). This model postulates that lizards should engage in thermoregulation only when the costs incurred do not outweigh the benefits. However, some evidence suggests that under thermal constraint, ectotherms will compensate by means of behavioral or physiological strategies, thus exhibiting higher efficiency of thermoregulation (Herczeg et al. 2003; Besson and Cree 2010; Aguado and Braña 2014).

In this context, some studies suggest that thermal preferences can change rapidly among populations and species (Angilletta et al. 2002). This labile response can be associated with phenotypic plasticity or adaptive response to environmental conditions (West-Eberhard 1989). In some cases, to compensate for harsh environments, natural selection acts on key parameters of organisms’ thermal biology, such as active body temperature ($T_b$), preferred body temperature ($T_p$: thermal preferences calculated in an environment that is independent of the ecological costs and constraints), and minimum and maximum critical temperatures ($CT_{min}$, $CT_{max}$: when the locomotor response of organisms is lost). Some ectotherms inhabiting harsh environments can even be thermoconformers (Huey and Slatkin 1976; Van Damme et al. 1989; Hertz et al. 1993; Angilletta et al. 2002; Angilletta 2009). These physiological adaptations could favor individuals inhabiting low-temperature environments (Somero 2010; Huey et al. 2012; Cahill et al. 2013). However, in other cases, it appears that thermal physiology does not evolve readily and thermal requirements are conservative among different populations and species (Hertz et al. 1983; Crowley 1985; Andrews 1998; Seebacher and Shine 2004; Díaz de la Vega-Pérez et al. 2013). In fact, closely related species inhabiting contrasting environments show similar thermal requirements, producing different thermoregulatory
costs (Bogert 1949; Hertz et al. 1983; Crowley 1985). In this case, the organisms must compensate for low thermal quality by highly efficient thermoregulatory behavior and/or physiological or morphological strategies (Díaz 1997; Ashton and Feldman 2003). Currently, the conservative and locally adaptive/acclimation responses are considered two ends of a continuum of evolutionary patterns (Angilleta et al. 2002; Sagonas et al. 2013).

Although lizards within the same population are generally exposed to similar environments, they do not always encounter the same microclimatic conditions. Different thermal responses can also be observed depending on body size, body condition, coloration, or sex (Stevenson 1985; Atkinson 1994; Cullum 1998; Clusella-Trullas et al. 2009). In addition, reproductive individuals, especially females, often differ in their thermoregulatory behavior from non-reproductive individuals, since embryonic development requires certain thermal intervals (Georges et al. 2005). Lower body temperatures of viviparous pregnant females of the lizard genus *Sceloporus* are associated with the successful maturation of offspring, whereas higher temperatures can lead to embryonic death (Beuchat 1988; Andrews et al. 1997; Andrews and Schwarzkopf 2012; López-Alcaide et al. 2017). This implies that some reproductive stages are more sensitive to changes in temperatures and, in species with conservative thermal biology inhabiting sites with variable thermal quality, this constraint could increase the extinction risk of populations under climate warming (Sinervo et al. 2010). The lizards of the genus *Sceloporus* are conservative in their thermal requirements (Andrews 1998), even though they have a wide latitudinal and altitudinal distribution in North and Central America, from Washington State in the northwestern U.S. to Panama, and from 0 to more than 4600 m asl (Sites et al. 1992).
In the present study, we investigated the thermal biology of the lizard *Sceloporus grammicus* Wiegmann, 1828 in response to contrasting high-mountain environments along a 1550 m asl elevational gradient. Our study sites were three populations at 2600, 3100, and 4150 m asl on the slopes of La Malinche Volcano, Tlaxcala, Mexico. We compared the *de*, *Tb*, *Tp*, thermal accuracy (*db*), and efficiency of thermoregulation (*E* and *B-W* indexes) between sexes and reproductive stages, among and within populations following the methods of Hertz et al. (1993) and Blouin-Demers and Weatherhead (2001). As thermal requirements are considered conservative in the genus *Sceloporus* (Andrews 1998), we expected that key thermal parameters such as *Tb* and *Tp* would be similar among the three populations, despite variation in the thermal quality of the habitats along the altitudinal gradient (we expected higher sites to have lower thermal quality). In addition, we expected higher thermoregulation indexes in the populations inhabiting lower thermal quality sites. Finally, because reproductive status can influence lizards’ thermal biology, we expected that pregnant females would show different thermal requirements from non-pregnant conspecifics and be more thermally efficient.

**Materials and methods**

**Study species and sites**

*Sceloporus grammicus* is the most widely distributed lizard species in Mexico. It is a common species in scrub, mountainous, and urban habitats from southern Texas, USA to central Mexico. This lizard can be found at elevations from 2000 to more than 4600 m asl on the slopes of several volcanoes in Mexico (Sites et al. 1992; Lemos-Espinal and Ballinger 1995a, b). It is an insectivorous lizard with year-round diurnal activity. This lizard is viviparous and exhibits group-living and social interactions during the
reproductive season, which begins in autumn and lasts until young are born in the spring (Jiménez-Cruz et al. 2005; Durán-Servín 2012). On La Malinche volcano, S. grammicus can be found from 2300 to 4460 m asl. This volcano is located within a natural protected area: “La Malinche National Park” (LMNP), Mexico.

**Fieldwork**

We followed the Official Mexican Norm NOM-126-ECOL-2000 as a guideline to handle the lizards under the collecting permits: SGPA/DGVS/15396/15 and SGPA/DGVS/007736/18. The lowest study site was at 2600 m asl (19°14´43.5´´ N, 97°55´36.0´´ W; Datum WGS 84). At this site, the lizards inhabit the rock walls of an abandoned hacienda (San Cristobal) and small isolated *Quercus* trees, surrounded by cornfields. The middle population, located at 3100 m asl (19°14´27.7´´ N, 97°58´46.6´´ W), was near La Malinche Scientific Station (SSM); this area is a grassland surrounded by *Abies religiosa* (Kunth), Schltldl. and Cham. and *Pinus montezumae* Lamb, forest. At this site, the lizards occupy fallen trees, boulders, and rock walls. The highest population was near the top of the volcano at 4150 m asl (19°14´24.7´´ N, 98°02´04.1´´ W), in a rocky alpine grassland above the tree line. The lizards inhabit natural volcanic rocks and small cedar shrubs (*Juniperus monticola* Martínez). All the study sites were polygons of ~4 ha on the east slope of the volcano.

Fieldwork was performed during 42 months (Sep-2014 – Feb-2018). All the study sites were visited at least 8 times per year to collect thermoregulatory data from different seasons and reproductive stages of the lizards. We captured lizards during their daily activity period (from 8:00 to 18:00 hours). Active lizards were captured by hand or by noose. Immediately after capture, we recorded *Tb* inside lizards’ cloacae, air temperature
(shaded sensor 5 cm above the substrate, $T_a$), and substrate temperature (shaded sensor in contact with the substrate, $T_s$) at the perch site with a digital quick reading thermometer (Fluke 52 II). All lizards that required more than 2 minutes from first sighting to capture were excluded from the analysis of $T_b$.

The lizards were transferred to La Malinche Scientific Station for studies on thermal physiology. We determined sex by the presence (males) or absence (females) of enlarged post-cloacal scales, reproductive condition (pregnant or non-pregnant) by palpation, snout-vent length (with a transparent ruler in mm, +/- 1mm, SVL), total length (in mm, +/- 1mm, TL), and body mass (in g, using a 10g, +/- 1g, Pesola® spring scale). We marked captured lizards by toe-clipping (Medica et al. 1971) to avoid repeated measures of the same individuals. We used Kwik-Stop® styptic powder after toe-clipping to avoid infections.

**Field operative temperatures ($T_e$)**

We used biophysical models to determine the range of potential body temperatures available to lizards in the absence of thermoregulatory behavior (Bakken 1992; Hertz et al. 1993; Peterson et al. 1993). These models were cylindrical hollow polyvinylchloride (PVC) pipes with the ends sealed with cork and painted with gray primer. All models were of similar shape and size (7 cm long x 1.5 cm wide) to *S. grammicus*. The PVC models were previously calibrated under field conditions during the activity period of the lizards following Adolph (1990), Sinervo et al. (2010), Díaz de la Vega-Pérez et al. (2013) and Lara-Resendiz et al. (2014).

Activity periods were determined according to the first and last field sightings from each site. Two biophysical models of lizards were connected to a data logger (HOBO pro v2-U23-003) with two sensor channels. In total, four models were placed to record $T_e$ at
each study site. Recording took place during Jan-2015 – Feb-2018 (539 days) at the 2600 m asl site, Dec-2014 – Feb-2018 (890 days) at the 3100 m asl site, and Feb-2015 – Feb-2018 (1096 days) at the 4150 m asl site. The variation in how long $Te$ was recorded among sites occurred because data loggers at the lower and middle sites were damaged and replaced.

To characterize the thermal environment, the PVC models were placed in typical perch sites under different sun light conditions (sun, shade, and mosaic) used by lizards. Additionally, at all study sites we changed the models to different perch sites during the three years we recorded data to randomize the microclimatic characteristics. The data loggers recorded the internal temperature of the biophysical model at intervals of 30 minutes. For our analyses, only the measurements taken during the lizards’ diurnal activity period observed in field were used to estimate the $Te$ at each study site.

**Laboratory work and thermoregulatory indices**

**Preferred body temperature**

Only adults were used for our laboratory tests and the cutoff SVL at which we considered lizards to be adults varied by site (2600 m asl: > 44 mm SLV; 3100 m asl: > 39 mm SLV and 4150 m asl: > 39 mm SLV) due to previous work suggesting differences in body size at sexual maturity according to elevation (Lemos-Espinal and Ballinger 1995b; Lemos-Espinal et al. 1998; Jiménez-Cruz et al. 2005). $Tp$ was determined under controlled laboratory conditions in a thermal gradient that consisted of a polycarbonate terrarium (1.2 m wide x 1.8 m long x 50 cm high) separated into 15 cm wide tracks. We suspended six 150 W bulbs 50 cm above one end of the terrarium to generate a thermal gradient from 10 to 45 ºC. Cold white lights were also suspended above the entire length of the terrarium to avoid differences in light intensity. The substrate was clean peat moss, and small rocks and
small logs were placed as typical perch sites along the tracks. However, we prevented the lizards from hiding under the perch sites by filling gaps with substrate. We included perch sites in the thermal gradient because *S. grammicus* is a rock-dwelling/arboreal and thigmothermic lizard, so providing perch sites allows lizards to avoid stress and exhibit normal thermoregulatory behavior (Díaz de la Vega-Pérez et al. 2013). Body temperature data in the thermal gradient were collected by manually capturing the animal and measuring body temperature in the cloaca, using a digital thermometer (Fluke 52 II), every 90 minutes between 9:00 to 18:00 h. The lizards were allowed to acclimate in the thermal gradient for one hour before the first temperature measurement. We provided water *ad libitum* before and fed the lizards two mealworms after the test. Laboratory tests were carried out one day after capture.

*Tb, Te, and Tp* were used to calculate *de, db*, and the thermoregulatory efficiency index (*E*) following the protocol of Hertz et al. (1993), and the thermoregulatory effectiveness index (*B-W*) proposed by Blouin-Demers and Weatherhead (2001) (see Appendix). Some controversy exists on the use of the Hertz et al. (1993) thermal efficiency index, because the same value of *E* can result from a variety of different combinations of *db* and *de* (i.e., mathematical origin of a rate, *db/de*; for details see Hertz et al. 1993). However, Blouin-Demers and Weatherhead (2001) proposed a different method to determine the efficiency of thermoregulation, this method determines the magnitude of the difference between *de* and *db* indexes (*de – db*) as a measure of how much an animal departs from thermoconformity. In general, the use of both indexes improves our understanding of the efficiency of thermoregulation and allows to compare our results with previous studies. The standard errors of *E* and *de* for each population were calculated by
bootstrapping. For the $E$ indexes, we used the mean $db$ for each population and 1000 iterations of $de$ (Hertz et al. 1993). We then compared $E$ and $de$ values among populations based on the bootstrapping estimates of their 95% confidence intervals.

**Thermal tolerances**

Laboratory tests of $CT_{\text{min}}$ and $CT_{\text{max}}$ were performed to determine physiological tolerances of the lizards from our three focal populations. Twenty lizards (10 males and 10 non-pregnant females) from each population were used to determine thermal tolerances, with 5 individuals of each sex used to determine $CT_{\text{min}}$ and 5 individuals of each sex used to determine $CT_{\text{max}}$. To determine $CT_{\text{min}}$ and $CT_{\text{max}}$, each lizard was placed in a dry plastic container (27 x 16 x 18 cm) surrounded by ice or under a lamp (150 W), respectively. In the case of $CT_{\text{min}}$ the temperature inside of the container decreased during contact with the ice. The starting $Tb$ of the lizard was 23 °C, then temperature was slowly decreased or increased (1.5 °C/min and 2.5 °C/min respectively) to determine $CT_{\text{min}}$ or $CT_{\text{max}}$. Every minute $Tb$ was recorded (with a digital thermometer, Fluke 52 II) inside lizards’ cloacae and the lizard was turned on its back. The temperature at which the lizard could not right itself and had lost the capability to move was considered the critical temperature ($CT_{\text{min}}$ for the lower limit and $CT_{\text{max}}$ for the upper limit). At the end of the test, the lizards were removed immediately from the cooled/heated container and allowed to recover in a container at room temperature (20 °C). Each lizard was used only once during this experiment, and no lizard died. Pregnant females were not used in this test because we did not want to compromise the development of the embryos or the health of the females (Beuchat 1988). All lizards were released at their place of capture after the experiments.

**Statistical analysis**
We analyzed and compared $T_b$, $T_p$, $T_e$, thermoregulation indexes ($de$, $db$, $E$), $CT_{\text{min}}$, and $CT_{\text{max}}$ among adult lizards from our three focal populations. Assumptions of normality and homogeneity of variances were analyzed using the Kolmogorov-Smirnov and Levene’s tests, respectively. When data satisfied the requirements for parametric tests, we used Student’s t-test or Analysis of Variance (ANOVA); otherwise non-parametric Kruskal-Wallis tests were used. The significance value in all statistical tests was $P < 0.05$, post hoc pair-wise comparisons were implemented to test for significant differences using the Tukey test (parametric) or Dunn’s method (non-parametric). We tested by Spearman correlation the $T_b$ vs $T_s$ and $T_a$ to determine whether lizards in each population exhibited thigmothermy or heliothermy. We determined the standard deviation of $E$ for each population by bootstrapping, using the average $db$ from each population and 1,000 simulations of $de$. We used generalized linear models (GLM) with paired contrast effects to compare $T_b$ and $T_p$ within and among populations, with elevation (2600, 3100, and 4150 m asl), sex, and reproductive condition (males, non-pregnant females, and pregnant females) as factors. The statistical analyses were performed and graphics were made with SigmaPlot version 11 (Systat Software, San Jose, CA 2008) and JMP version 13.1.0 (SAS 2016). Bootstrap was performed with “boot” package and was conducted in R Studio software (R Development Core Team 2012).

**Results**

We recorded at least three full years of temperature data at all study sites. The activity period of lizards from the middle altitude (8:00 to 17:00 h, $n = 385$) was different ($H = 27.01$, $d.f. = 2$, $P < 0.001$) to the lowest and highest altitude sites (9:00 to 18:00 h, $n = 385$;
10:00 to 18:00 h, \( n = 323 \), respectively), but the lowest and highest altitude sites did not differ from each other (Fig. 1).

**Body temperatures (\( Tb \))**

The lizards from 2600 m asl exhibited the narrowest range of \( Tb \) \((H = 117.90, d.f. = 2, P < 0.001)\), from 17 to 38 °C \((n = 344)\), meanwhile the lizards from 3100 m asl were active at body temperatures from 13 to 37 °C \((n = 179)\), similar to the lizards from 4150 m asl that ranged from 12 to 38 °C \((n = 218)\). In contrast, the average \( Tb \)'s of lizards were different among the three populations \((x^2 = 76.082, d.f. = 2, P < 0.001)\), with the lowest \( Tb \) in lizards from the highest population \((27.4 °C)\), and higher \( Tb \)'s in the middle and the lowest populations \((29.7 °C\) and \(31.5 °C, \) respectively).

We tested for effects of the interaction of sex and reproductive condition on \( Tb \) variation among populations, and we observed that males \((x^2 = 37.093, d.f. = 2, P < 0.001, n = 324)\) and pregnant females \((x^2 = 28.995, d.f. = 2, P < 0.001, n = 56)\) from the highest population had significantly lower \( Tb \) than the other two populations, but there were no differences between the latter two. In contrast, non-pregnant females from the lowest site had significantly higher \( Tb \) than the middle and the highest populations, but there were no differences between these latter two \((x^2 = 41.652, d.f. = 2, P < 0.001, n = 351)\) (Table 1).

In the within population comparisons, we found an effect of gestation on female \( Tb \) in the highest population: pregnant females had significantly lower \( Tb \) than males and non-pregnant females lizards \((4150\ m\ asl\ : x^2 = 16.079, d.f. = 2, P < 0.001)\). However, \( Tb \) from the lowest and middle populations did not significantly differ between the sexes or between pregnant and non-pregnant females \((2600\ m\ asl\ : x^2 = 1.478, d.f. = 2, P = 0.224; 3100\ m\ asl: x^2 = 2.117, d.f. = 2, P = 0.145)\) (Table 2).
The lizards from all populations use a thigmothermy strategy, because the \( T_b \) were more closely correlated with \( T_s \) than with \( T_a \) (2600 m asl: \( T_b \) vs \( T_a \): \( r = 0.28, P < 0.001, n = 330 \); \( T_b \) vs \( T_s \): \( r = 0.34, P < 0.001, n = 330 \); 3100 m asl: \( T_b \) vs \( T_a \): \( r = 0.35, P < 0.001, n = 176 \); \( T_b \) vs \( T_s \): \( r = 0.40, P < 0.001, n = 176 \); 4150 m asl: \( T_b \) vs \( T_a \): \( r = 0.39, P < 0.001, n = 215 \); \( T_b \) vs \( T_s \): \( r = 0.48, P < 0.001, n = 215 \)).

**Preferred body temperature (T\(_p\))**

We tested for an interaction effect of sex and reproductive condition on \( T_p \) among populations, and we found that males (\( \chi^2 = 22.002, d.f. = 2, P < 0.001, n = 264 \)) and non-pregnant females (\( \chi^2 = 19.937, d.f. = 2, P < 0.001, n = 325 \)) from the highest population had significantly higher \( T_p \) than the other two populations (Fig. 2a, b). In the case of pregnant females, we did not find statistical differences among them (\( \chi^2 = 2.091, d.f. = 2, P = 0.148, n = 139 \)) (Fig. 2c) (Table 1).

Similar to our \( T_b \) results, we found an effect of gestation on \( T_p \) in all intrapopulation comparisons. Within populations, pregnant females exhibited lower \( T_p \), regardless of their population of origin (2600 m asl: \( \chi^2 = 59.091, d.f. = 2, P < 0.001 \); 3100 m asl: \( \chi^2 = 23.240, d.f. = 2, P < 0.001 \); 4150 m asl: \( \chi^2 = 72.650, d.f. = 2, P < 0.001 \)), but the \( T_p \) of males and non-pregnant females from the three populations did not show significant differences (Table 2).

Furthermore, the \( T_b \) of the lizards from the highest population is lower than \( T_p \) for both sexes and reproductive conditions (\( \chi^2 = 113.643, d.f. = 1, P < 0.001 \)). Meanwhile, in the middle population, no differences were observed (\( \chi^2 = 0.015, d.f. = 1, P = 0.90 \)), and at the lowest site only non-pregnant females exhibited differences (\( \chi^2 = 8.391, d.f. = 1, P = 0.003 \)) between \( T_b \) and \( T_p \) (Fig. 3).
Thermal tolerances

It should be noted that $CT_{\text{min}}$ and $CT_{\text{max}}$ were found outside the activity $Tb$ of the lizards from all populations (Fig. 4a, b, c). Lizards from the highest population had significantly lower $CT_{\text{min}}$ (9.2 °C) than those from the two lower populations ($F_{2, 27} = 19.59, P < 0.001$); meanwhile, the $CT_{\text{max}}$ was different only between the middle (41.2 °C) and highest (40 °C) populations ($F_{2, 27} = 4.83, P = 0.01$). However, we did not find significant differences in $CT_{\text{min}}$ or $CT_{\text{max}}$ between sexes within any of our three study populations (Table 3).

Operative temperatures ($Te$)

The PVC models accurately mimicked the thermal characteristics of the lizards ($r = 0.82, P < 0.001, n = 59$). The $Te$ was statistically different among all populations ($H = 43580.97, d.f. = 2, P < 0.001, n = 203,549$), with the highest study site presenting the lowest $Te$ (Fig. 4d, e, f).

Thermoregulation indexes

The $de$ indexes differed significantly among all populations ($F_{2, 2997} = 51623.73, P < 0.001$): the highest study site had the highest value of $de$ (i.e. lower thermal quality), the medium study site had moderate thermal quality, and the lowest site had the lowest value of $de$ (i.e. highest thermal quality) (Fig. 5a) (Table 1). We tested the effect of season on thermal quality within each study site; winter had the lowest $de$ at the high- and middle-altitude sites (4150 m asl: $H = 1689.08, d.f. = 3, P < 0.001, n = 77,921$; 3100 m asl: $H = 1528.87, d.f. = 3, P < 0.001, n = 43,437$) due to the low environmental temperatures; meanwhile, in the lowest altitude population autumn had the lowest $de$ (2600 m asl: $H = 5223.13, d.f. = 3, P < 0.001, n = 33,787$). Similarly, $db$ indexes were different among populations ($H = 82.72, d.f. = 2, P < 0.001, n = 741$), increasing with the population’s elevation (Table 1).
lizards from the lowest altitude population thus had the lowest $db$ index (i.e. higher thermoregulatory accuracy).

The $E$ index differed significantly among populations ($H = 2664.31$, $d.f. = 2$, $P < 0.001$, $n = 3,000$). Lizards from the lowest population thermoregulated more efficiently ($E = 0.92 \pm 0.005$), than the lizards from the two higher populations (3100 m asl: $E = 0.89 \pm 0.006$; 4150 m asl: $E = 0.82 \pm 0.006$) (Fig. 5b). Similar to the $E$ index, the $B-W$ indexes were different among populations with the tendency to increase with elevation: the lizards from the highest population had the highest $B-W$ index with 15.6 °C, suggesting they compensate for the lowest $de$ by active thermoregulation (Table 1). The middle population had a $B-W$ index of 10.8 °C and the lowest population had a $B-W$ index of 8.6 °C.

**Discussion**

*Sceloporus grammicus* is a widely distributed lizard species that inhabits a variety of ecosystems, mainly in Mexico. It is a well-studied species; however, here we document for the first time its thermal ecology and the relation between this species’ thermal requirements (i.e. $Tp$), the thermal characteristics of its habitat, and its thermal efficiency indexes along an elevation gradient in a high mountain system.

In the high-mountain ecosystem La Malinche, the influence of elevation on *S. grammicus* body temperatures was evident, principally in the comparisons of the extreme sites along the altitudinal gradient. In support of our first prediction, we found that the influence of elevation on the thermal environment was significant and generate lower thermal quality sites at higher elevations. This result supports previous work, which evaluated the thermal quality of the environment of nine species of lizards from an altitudinal gradient; they observed that besides the altitude, the type of vegetation, and
coverage influences this index (Lara-Resendiz et al. 2014). The lizards from the highest site presented more variable body temperatures and the lowest average ($T_b = 27.6 \, ^\circ\text{C}$). In fact, among lizards of the same sex or reproductive condition, lizards from the highest population always had lower body temperature, because it is influenced by the thermal environment and lizards from the highest elevation population are exposed to the lowest thermal quality. Nevertheless, this is not a general pattern regarding how lizards from high elevations differ in thermoregulatory behavior from lizards living at low or intermediate elevations (Zamora-Camacho et al. 2016). For example, Lemos-Espinal and Ballinger (1995a) previously found that $S.$ grammicus inhabiting mountains at high elevations (4400 m) had high body temperature (31.5 °C), similar to those that we found at lower sites. These differences could be associated with the thermal quality in the high-elevation mountains, it is possible that at the microclimatic scale, there could exist different thermic environments that impose different costs. For this reason, it is important to determine accurately the thermal quality of these extreme sites. On the other hand, the body temperature from the two lowest populations were similar to those previously recorded for $S.$ grammicus at similar elevations (see Woolrich-Piña et al. 2006; Lara-Resendiz and Díaz de la Vega-Pérez 2013).

Sex and reproductive condition in $Sceloporus$ have previously been shown to affect individuals’ thermal requirements. In some cases, $Sceloporus$ females exhibit lower body temperatures during pregnancy, compared to non-pregnant females and males (Andrews et al. 1997; Woolrich-Piña et al. 2006). Here, only pregnant females from the highest population exhibited reduced body temperature (22.2 °C), similar to previously results from Woolrich-Piña et al. (2006) but at low elevations (2240 m asl). This could be because
pregnant females confront limitations or restrictions on their thermoregulation; for that reason, differences in body temperature are present only in the site with lowest thermal quality, where thermoregulation is most costly. Also, it is widely known that the body temperature during gestation influences the duration and direction of embryonic development in reptiles: lower body temperature could mean longer gestation periods, but higher body temperature could negatively affect the embryos’ later growth, behavior, locomotor performance, and survival (Beuchat 1988; Andrews et al. 1997; Woolrich-Piña et al. 2006).

The thermal preferences among lizard populations were similar, despite their inhabiting environments with different thermal restrictions. These observations agree with previous reports in the genus *Sceloporus* (Adolph 1990; Lemos-Espinal and Ballinger 1995a; Andrews 1998). The narrow thermal requirements of the species constrain the activity of the lizards and could favor different behavioral, physiological, morphological, and ecological strategies that allow them to inhabit thermically harsh sites. These results are consistent with previous observations in *S. grammicus* (Lara-Resendiz and Díaz de la Vega-Pérez 2013) and with the conservative thermal requirements hypothesis in the genus *Sceloporus* (Andrews 1998). Also, this conservative pattern of thermal preferences among populations of the same species, has been observed in other genera of lizards, including populations found in different habitats and thermal conditions (Marquet et al. 1989; Díaz de la Vega-Pérez et al. 2013). Interestingly, pregnant females from all populations selected similar low thermal preferences (< 30 °C) regardless of the thermal quality of their habitat. This result agrees with previous observations in pregnant females of the genus *Sceloporus* (Beuchat 1988; López-Alcaide et al. 2017). Reproduction imposes costs and tradeoffs that
could be higher for individuals inhabiting low thermal quality habitats. Also, pregnant females are often more prone to predation than males or non-pregnant females, since greater body mass produces lower locomotor performance (Shine 2003). However, this result is not universal (Zamora-Camacho et al. 2014a).

We found significant differences in thermal efficiency indexes among populations, which indicates the thermal pressures and lizards’ responses to them varied among sites. The lizards from 4150 m asl had the lowest $E$ index (0.82), thermal quality (18.93), and accuracy (3.27) and must compensate $\sim$15.6 °C to thermoregulate according to the $B-W$ index. Most importantly, the environmental temperature from this site is close to the $CT_{min}$. In spite of all these constraining thermal factors, the lizards are abundant. *Sceloporus grammicus*, like other lizards, are constrained by their thermal range in where they can carry out their physiological processes in an optimal way (Woolrich-Piña et al. 2006). The $CT_{min}$ and $CT_{max}$ from the three populations are within the recorded ranges of the operative temperatures; indeed, the averages of operative temperatures and $CT_{min}$ (10.5 °C and 9.2 °C, respectively) from the highest population are similar. This means that lizards from this population are inhabiting restricted thermal conditions and are highly efficient thermoregulators according to $B-W$ index. Although *S. grammicus* makes use of both thigmothermy and heliothermy (Woolrich-Piña et al. 2006), we observed in our three populations that the most used strategy for thermoregulation is thigmothermy, probably because substrate temperature could be higher than air temperature in the high mountain system they inhabit. In addition, low thermal quality values could affect lizards’ physiological traits, such as reproduction, metabolism, growth, locomotor performance, and also behavior (Crowley 1985; Lemos-Espinal and Ballinger 1995a, b; Andrews et al. 1997).
Some strategies to compensate low temperatures may be morphological, as they have smaller bodies, which could help to gain heat faster (Clusella-Trullas et al. 2009). Nevertheless, other environmental factors or natural selection pressures could modify body sizes of lizards (Jiménez-Arcos et al. 2017). Further work is necessary to evaluate the impact of environmental temperature on body size, whether smaller bodies reach optimal temperatures in short time periods, and how local adaptation in body size affects other life-history traits (Ashton and Feldman 2003; Zamora-Camacho et al. 2014b).

Thermal physiological constraint (stenothermy) may increase lizards’ vulnerability in the face of rising environmental temperatures due to climate change (Sinervo et al. 2010). Nevertheless, species as *S. grammicus* that have a wide repertory of strategies to efficiently achieve their thermal requirements when inhabiting harsh environments could be a survivor in this catastrophic current event. More evidence of the thermal efficiency of widely distributed lizard species inhabiting high mountain ecosystems that are exposed to diverse thermal environments is need to accurately calculate their vulnerability under climate change scenarios.

In summary, our research produced six main conclusions: 1) we found that the altitudinal gradient in a high-mountain system determines the thermal quality of the sites and affect the body temperature of the lizards; 2) the lizards from the highest site must compensate more than 15 °C to achieve their thermal requirements; 3) nevertheless, thermal requirements are conserved, and the lizards must compensate for harsh environments by thermoregulatory strategies; 4) we recorded for the first time in *S. grammicus* a relationship between pregnancy, the selection of low thermal preferences, and more accurate thermoregulation than non-pregnant females or males; 5) these data support
conservative thermal requirements in the genus Sceloporus and do not agree with the cost-benefit model; 6) a wide repertoire of strategies enables S. grammicus to inhabit a wide latitudinal and altitudinal range distribution, being an ecological generalist and a successful species.

Acknowledgements

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References


Available from


Systat Software, San Jose, CA 2008. SigmaPlot version 11. Available from


**Figure legends**

**Figure 1.** Activity period of Mesquite lizard, *Sceloporus grammicus* Wiegmann, 1828, at the three different altitudes in La Malinche Volcano National Park.

**Figure 2.** Average of preferred body temperature in the laboratory (*Tp*) (mean ± SD) of Mesquite lizard (*Sceloporus grammicus* Wiegmann, 1828): males (a), non-pregnant females (b), pregnant females (c) among populations. Capital letters denote statistical differences.

**Figure 3.** Field body temperatures (*Tb*) and preferred body temperature in the laboratory (*Tp*) of Mesquite lizard, *Sceloporus grammicus* Wiegmann, 1828, by sex and reproductive condition (males, non-pregnant females, and pregnant females) and altitude (4150 (a), 3100 (b) and 2600 (c) m asl). The black points represent *Tb* and white squares *Tp* (mean ± SD). Capital letters denote statistical differences between thermal parameters.

**Figure 4.** Distribution of field body temperatures (*Tb*) of Mesquite lizard (*Sceloporus grammicus* Wiegmann, 1828): 4150 m asl (a), 3100 m asl (b), 2600 m asl (c) and operative temperature (*Te*): 4150 m asl (d), 3100 m asl (e), 2600 m asl (f) at the study sites. The shaded area indicates the interquartile *Tset* range for each population in the thermal...
gradient and the arrow indicates the average of $T_p$, the dotted line indicates the $CT_{max}$ and the dashed line indicates the $CT_{min}$ determined for each population. Notice the difference of scales.

**Figure 5.** Thermal quality index of the habitat ($de$) (Fig. 5a) and thermoregulatory efficiency index (Fig. 5b) of the Mesquite lizard (*Sceloporus grammicus* Wiegmann, 1828) at each altitude in the La Malinche Volcano National Park. For $de$ the points represent the mean and the upper and lower bar the standard deviation (mean ± SD) Capital letters denote statistical differences. The $E$ indexes were calculated by bootstrapping, using mean $db$ for each population and 1000 iterations of $de$. The box represents the median and the first and third quartile, and the whiskers indicate the minimum and maximum values. Capital letters denote statistical differences.
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163x314mm (300 x 300 DPI)
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173x323mm (300 x 300 DPI)
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Table 1. Thermoregulatory and morphometric variables in *Sceloporus grammicus* Wiegmann, 1828, at three study sites. Mean ± Standard deviation are given, with sample size \( (n) \) in parentheses. Lowercase letters denote significant statistical differences among populations.
<table>
<thead>
<tr>
<th>Variables/Sites</th>
<th>2600 m asl</th>
<th>3100 m asl</th>
<th>4150 m asl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tb (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>31.4 ± 3.6 (159)a</td>
<td>29.3 ± 5.0 (59)a</td>
<td>27.9 ± 5.1 (108)b</td>
</tr>
<tr>
<td>Non-pregnant females</td>
<td>32.1 ± 2.5 (168)a</td>
<td>29.6 ± 3.6 (88)b</td>
<td>27.9 ± 5.5 (97)b</td>
</tr>
<tr>
<td>Pregnant females</td>
<td>29.3 ± 3.4 (17)a</td>
<td>30.4 ± 3.8 (32)a</td>
<td>22.2 ± 4.2 (13)b</td>
</tr>
<tr>
<td>Population</td>
<td>31.6 ± 3.2 (344)a</td>
<td>29.7 ± 4.1 (179)b</td>
<td>27.5 ± 5.1 (218)c</td>
</tr>
<tr>
<td><strong>Tp (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>30.8 ± 4.7 (96)a</td>
<td>30.4 ± 4.4 (53)a</td>
<td>31.8 ± 3.5 (115)b</td>
</tr>
<tr>
<td>Non-pregnant females</td>
<td>30.4 ± 4.3 (121)a</td>
<td>30.2 ± 4.8 (100)a</td>
<td>31.5 ± 3.4 (104)b</td>
</tr>
<tr>
<td>Pregnant females</td>
<td>27.9 ± 5.6 (45)a</td>
<td>28.9 ± 3.6 (44)a</td>
<td>29.0 ± 4.3 (50)a</td>
</tr>
<tr>
<td>Population</td>
<td>30.1 ± 4.6 (262)a</td>
<td>30 ± 4.5 (197)a</td>
<td>31.1 ± 3.7 (269)b</td>
</tr>
<tr>
<td><strong>Interquartile range (Tp25-Tp75; °C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>28.5-34.1 (96)</td>
<td>28.7-33.2 (53)</td>
<td>30.5-34.1 (115)</td>
</tr>
<tr>
<td>Non-pregnant females</td>
<td>28-33.4 (121)</td>
<td>27.5-33.7 (100)</td>
<td>29.6-33.9 (104)</td>
</tr>
<tr>
<td>Pregnant females</td>
<td>25.7-31.5 (45)</td>
<td>28.4-30.9 (44)</td>
<td>28.6-31.5 (50)</td>
</tr>
<tr>
<td><strong>Thermoregulation indices</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermoregulatory accuracy (db)</td>
<td>0.72</td>
<td>1.28</td>
<td>3.24</td>
</tr>
<tr>
<td>Habitat thermal quality (de)</td>
<td>9.35a</td>
<td>12.13b</td>
<td>18.93c</td>
</tr>
<tr>
<td>Thermoregulatory efficiency index (de-db)</td>
<td>8.6</td>
<td>10.8</td>
<td>15.6</td>
</tr>
<tr>
<td>Thermoregulatory efficiency index (E)</td>
<td>0.92 ± 0.005a</td>
<td>0.89 ± 0.006b</td>
<td>0.82 ± 0.006c</td>
</tr>
<tr>
<td><strong>Snout-vent length (SVL; mm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>64 ± 8.4 (187)</td>
<td>63 ± 8.9 (83)</td>
<td>50 ± 4.7 (140)</td>
</tr>
<tr>
<td>Females</td>
<td>57 ± 7.2 (233)</td>
<td>56 ± 6.5 (145)</td>
<td>48 ± 4.2 (154)</td>
</tr>
<tr>
<td><strong>Body mass (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>7.8 ± 2.6 (171)</td>
<td>7.6 ± 3.0 (70)</td>
<td>3.9 ± 1.1 (131)</td>
</tr>
<tr>
<td>Non-pregnant females</td>
<td>5.3 ± 1.8 (195)</td>
<td>5.3 ± 2.2 (105)</td>
<td>3.6 ± 1.2 (133)</td>
</tr>
<tr>
<td>Pregnant females</td>
<td>5.9 ± 1.4 (21)</td>
<td>6.5 ± 2.0 (42)</td>
<td>3.7 ± 0.7 (23)</td>
</tr>
</tbody>
</table>
Table 2. Paired contrast effects comparing body temperatures ($T_b$) and preferred body temperature ($T_p$) within populations, with population (2600, 3100, and 4150 m asl), sex, and reproductive condition (males, non-pregnant females, and pregnant females) as factors in *Sceloporus grammicus* Wiegmann, 1828.

<table>
<thead>
<tr>
<th>Variables/Sites</th>
<th>2600 m asl</th>
<th>3100 m asl</th>
<th>4150 m asl</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_b$ Males vs Non-pregnant females</td>
<td>$x^2 = 0.666, d.f. = 1, P = 0.139$</td>
<td>$x^2 = 0.264, d.f. = 1, P = 0.700$</td>
<td>$x^2 = 0.084, d.f. = 1, P = 0.881$</td>
</tr>
<tr>
<td>$T_b$ Males vs Pregnant females</td>
<td>$x^2 = 2.079, d.f. = 1, P = 0.045$</td>
<td>$x^2 = 1.087, d.f. = 1, P = 0.245$</td>
<td>$x^2 = 5.690, d.f. = 1, P &lt; 0.001$</td>
</tr>
<tr>
<td>$T_b$ Non-pregnant females vs Pregnant females</td>
<td>$x^2 = 2.745, d.f. = 1, P = 0.008$</td>
<td>$x^2 = 0.822, d.f. = 1, P = 0.350$</td>
<td>$x^2 = 5.775, d.f. = 1, P &lt; 0.001$</td>
</tr>
<tr>
<td>$T_p$ Males vs Non-pregnant females</td>
<td>$x^2 = 0.354, d.f. = 1, P = 0.226$</td>
<td>$x^2 = 0.261, d.f. = 1, P = 0.501$</td>
<td>$x^2 = 0.372, d.f. = 1, P = 0.194$</td>
</tr>
<tr>
<td>$T_p$ Males vs Pregnant females</td>
<td>$x^2 = 2.914, d.f. = 1, P &lt; 0.001$</td>
<td>$x^2 = 1.486, d.f. = 1, P &lt; 0.001$</td>
<td>$x^2 = 2.809, d.f. = 1, P &lt; 0.001$</td>
</tr>
<tr>
<td>$T_p$ Non-pregnant females vs Pregnant females</td>
<td>$x^2 = 2.559, d.f. = 1, P &lt; 0.001$</td>
<td>$x^2 = 1.224, d.f. = 1, P = 0.003$</td>
<td>$x^2 = 2.436, d.f. = 1, P &lt; 0.001$</td>
</tr>
</tbody>
</table>
Table 3. Thermal tolerances $CT_{\text{min}}$ and $CT_{\text{max}}$ and effects of the interaction of sex (males and females) as factors at three study sites in *Sceloporus grammicus* Wiegmann, 1828. Mean of $CT_{\text{min}}$ and $CT_{\text{max}}$ by population. Sample size ($n$) in parentheses.

<table>
<thead>
<tr>
<th>Variables/Sites</th>
<th>2600 m asl</th>
<th>3100 m asl</th>
<th>4150 m asl</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CT_{\text{min}}$</td>
<td>12.3 °C (10)</td>
<td>10.0°C (10)</td>
<td>9.2 °C (10)</td>
</tr>
<tr>
<td>Males vs Females</td>
<td>$t = 0.0516$</td>
<td>$t = 0.466$</td>
<td>$t = 0.419$</td>
</tr>
<tr>
<td>$d.f.$</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>$P$</td>
<td>0.960</td>
<td>0.654</td>
<td>0.686</td>
</tr>
<tr>
<td>$CT_{\text{max}}$</td>
<td>40.3 °C (10)</td>
<td>41.2 °C (10)</td>
<td>40.0 °C (10)</td>
</tr>
<tr>
<td>Males vs Females</td>
<td>$t = 1.638$</td>
<td>$t = 0.001$</td>
<td>$t = 0.113$</td>
</tr>
<tr>
<td>$d.f.$</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>$P$</td>
<td>0.140</td>
<td>1.00</td>
<td>0.913</td>
</tr>
</tbody>
</table>
Appendix.

Efficiency of thermoregulation index according to Hertz et al. (1993).

To calculate $db$ we used the equation: $db = Tp_{(low\ limit)} - Tb$ when $Tb < Tp$, when $Tb > Tp$, then $db$ was calculated as: $db = Tb - Tp_{(high\ limit)}$. To calculate $de$ we used the equation: $de = Tp_{(low\ limit)} - Te$ when $Te < Tp$, when $Te > Tp$, then $de$ was calculated as: $de = Te - Tp_{(high\ limit)}$.

Only absolute values were used. When $Tb$ or $Te$ values were within the 50% range of the $Tp$ central interval, then $db$ and $de$ equaled zero. We calculated the mean temperatures at each study site to obtain $db$ and $de$. Values of $db$ and $de$ equal or close to zero indicate that environments are thermally ideal, while higher values indicate low thermal accuracy and quality.

The $E$ index was calculated using the equation $E = 1 - (db / de)$. The thermal efficiency index ranges from zero to one. A value close to one means the individuals within the population are active thermoregulators.

Efficiency of thermoregulation index according to Blouin-Demers and Weatherhead (2001). The $B-W$ index is defined as the difference between $db$ and $de$ to quantify the degree of departure from perfect thermoconformity. In this index, zero represents perfect thermoconformity, and a positive value represents animals that thermoregulate. For both indices ($E$ and $B-W$), a negative value represents the avoidance of thermally favorable habitats.

References