

RESEARCH ARTICLE

Impact of temperature on bite force and bite endurance in the leopard iguana (*Diplolaemus leopardinus*) in the Andes Mountains

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ABSTRACT

In ectotherms, temperature exerts a strong influence on the performance of physiological and ecological traits. One approach to understanding the impact of rising temperatures on animals and their ability to cope with climate change is to quantify variation in thermal-sensitive traits. Here, we examined the thermal biology, temperature dependence and thermal plasticity of bite force (endurance and magnitude) in *Diplolaemus leopardinus*, an aggressive and territorial lizard endemic to Mendoza province, Argentina. Our results indicate that this lizard behaves like a moderate thermoregulator that uses the rocks of its environment as the main heat source. Bite endurance was not influenced by head morphometry and body temperature, whereas bite force was influenced by head length and jaw length, and exhibited thermal dependence. Before thermal acclimation treatments, the maximum bite force for *D. leopardinus* occurred at the lowest body temperature and fell sharply with increasing body temperature. After acclimation treatments, lizards acclimated at higher temperatures exhibited greater bite force. Bite force showed phenotypic plasticity, which reveals that leopard iguanas are able to maintain (and even improve) their bite force under a rising-temperature scenario.

KEY WORDS: Global warming, Performance, Phenotypic plasticity, Reptiles, Thermal acclimation

INTRODUCTION

How rising temperatures affect organisms is of primary concern to enable predictions of the impact of the anthropogenic climate change on biota (Dawson et al., 2011). The Intergovernmental Panel on Climate Change (IPCC) predicts an increase of 1.5–2.0°C in mean global temperature over the next 20 years, as well as an increase in the frequency of heat waves, droughts and the frequency of extreme temperature events (IPCC, 2018). Under this scenario,

changes in phenology, abundance, distribution and extinction risk of natural populations are expected (Sinervo et al., 2010, 2018; Bestion et al., 2015; Llewelyn et al., 2018).

Ectotherms are especially vulnerable to climate change (Deutsch et al., 2008) because their performance depends on body temperature (T_b), which is largely dependent on environmental temperatures (Halliday and Blouin-Demers, 2017). Body temperature influences physiological performance including locomotion, bite force, reproduction, growth, courtship and activity patterns (Adolph and Porter, 1993; Anderson et al., 2008; Angilletta, 2009; Bestion et al., 2015; Kubisch et al., 2012). Typically, these traits are optimally efficient within a narrow range of T_b (Angilletta et al., 2002). Thus, the ability to maintain a relatively constant T_b , despite the thermally heterogeneous environment, has direct effects on survivorship and, therefore, fitness (Black et al., 2019).

Another strategy to cope with thermal heterogeneity includes the plasticity and adaptability of different traits (thermoregulatory behaviour, thermal sensitivity) to the changing abiotic circumstances (Angilletta, 2009; Black et al., 2019; Pigliucci, 2001). In particular, the potential of a genome to produce a range of phenotypes in response to distinct environmental conditions is known as phenotypic plasticity (Pigliucci, 2001). Thus, acclimation, consisting of reversible trait changes in response to environmental changes typically on a temporal scale of weeks or months, can be considered as a form of phenotypic plasticity (Schulte et al., 2011). The organism's ability to adjust thermally sensitive traits in a labile way, to the climate, allows predictions of population responses to global change (Llewelyn et al., 2018). However, this ability could vary across populations and species: some animals could maximize their performance at the temperature to which they are acclimated ('beneficial acclimation' hypothesis; Leroi et al., 1994; Wilson and Franklin, 2002), whereas other animals could perform better when they are acclimated to high temperatures than when they are acclimated to low temperatures ('hotter is better' hypothesis; Huey and Berrigan, 1996).

One of the performance traits that could be crucial for individual fitness is bite force (Anderson et al., 2008). Biting is used in prey selection (Erickson et al., 2004; Herrel and O'Reilly, 2006), prey handling (i.e. to capture, subdue and consume prey; Verwajen et al., 2002), defense against predators (Anderson et al., 2008) and aggressive interactions (Huyghe et al., 2005; Lailvaux et al., 2004), particularly as a key weapon in agonistic fights among males to determine dominance (Husak et al., 2006). Males also bite females during copulation, seemingly to restrain them (Herrel et al., 1996; Husak et al., 2006), which may prolong copulation and increase success. Bite force is strongly influenced by body size and proportions such as head shape and size (Herrel et al., 2001a). Compared with other performance traits such as locomotion, bite force is less dependent on temperature because muscular generation of force is relatively insensitive to thermal change (Bennett, 1985).

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Previous studies in *Trapelus pallida* (Herrel et al., 2007) and *Hemidactylus frenatus* (Cameron et al., 2018) showed that 80% of maximal performance of bite force could be sustained across a broad range of temperatures, 22.5 to 37.5°C for *T. pallida* and 20 to 30°C for *H. frenatus*, suggesting that bite force is largely independent from temperature in certain ranges. However, studies in several species of lizard, such as *Stellagama stellio*, *Uromastix aegyptia* and *U. acanthinura* (Herrel et al., 1999), *Gecko gecko* (Anderson et al., 2008) and *Bradypodion pumilum* (Segall et al., 2013), have shown that T_b affects bite force. Particularly, in *B. pumilum*, a steep drop in bite performance at higher T_b was observed. Thermal acclimation of bite force has been scarcely studied in ectotherms, thus the present study explores a novel field within the thermal biology of lizards.

Diplolaemus leopardinus, known as the leopard iguana, is endemic to high elevations in the Andes Mountains and lower elevations in the Patagonian steppe environments of Mendoza Province, Argentina. These lizards belong to the Leiosauridae family (Frost et al., 2001) and, like other leiosaurids, they are oviparous, insectivorous and occasionally saurophagous (Ceï, 1986; Donoso-Barros, 1966; Van Devender, 1977). Indeed, as in other allied genera, *Diplolaemus* species are also known as ‘gruñidores’ (grumblers), because both females and males produce a hissing sound during aggressive displays and they often vocalize and bite when disturbed (Laspiur et al., 2007; Donoso-Barros, 1966). The leopard iguana is a saxicolous (rock-dwelling), territorial lizard that is a sit-and-wait forager and uses self-made burrows typically under basking rocks. They are medium-sized lizards [mean snout–vent length (SVL): 104.5 mm], without evident sexual dimorphism or dichromatism, and are characterized by having a triangular head with strong wide jaws and marked development of the mandibular muscles (Ibargüengoytia et al., 2004; Victoriano et al., 2010; Fig. 1).

Given the ecological relevance of bite performance in *D. leopardinus* (diet, territorial and defensive behavior), we tested whether thermal variables influence bite force and bite duration, taking into account the expected direct effect of head morphology (Anderson et al., 2008; Herrel et al., 1999, 2001a; Verwaijen et al., 2002). Previous studies reported that bite force is influenced positively by head length and width, because a larger head may support both more muscle to generate bite force and longer bones for greater leverage (Herrel et al., 2001a), whereas bite endurance is influenced by lower jaw length (Gomes et al., 2020 preprint). We evaluated the thermal sensitivity of bite force and bite endurance before and after two thermal acclimation treatments under the hypothesis that variation

in temperature produces differences in bite performance. For the lizards analyzed before acclimation treatments, we predicted that maximum bite performance would be achieved at a T_b close to the species’ preferred temperatures, as shown for other physiological traits (i.e. locomotor performance; Angilletta et al., 2002), following the predictions of the ‘thermal coadaptation’ hypothesis (Angilletta et al., 2006; Huey and Bennett, 1987). For the lizards analyzed after acclimation treatments, we predicted (i) a plastic response in the thermal sensitivity of bite force, because leopard iguanas inhabit environments with great seasonal thermal variation, where maintenance of plasticity, despite its costs, would have a positive impact on fitness (Angilletta, 2009; Huey and Kingsolver, 1989; Pigliucci, 2001); and that (ii) those lizards acclimated to high temperatures should have better performance across all temperatures than those acclimated to low temperatures, following the ‘hotter is better’ hypothesis (Huey and Kingsolver, 1989; Huey and Berrigan, 1996).

MATERIALS AND METHODS

Study area

The study area was located in the Angostura mountain valley (32.983°S, 69.333°W, WGS84, 2405 masl), in west-central Mendoza Province, Argentina. This area is surrounded by mountain peaks more than 3000–4000 masl, and it is characterized by a cold arid climate with a mean annual temperature of 6.3°C and mean annual precipitation of 359 mm (Sileo et al., 2015). The study area registers maximum temperatures in summer of 29°C and minimum temperatures in winter of –15.5°C. In this type of environment, lizards remain inactive from autumn until early spring.

Fieldwork

Sampling was conducted 17–19 December 2018, in late spring. We captured by hand or noose 21 leopard iguanas (10 males and 11 females) during daylight, 09:00 to 18:00 h. We georeferenced each capture location using a handheld GPS (Garmin® eTrex 20, Garmin Ltd, Olathe, KA, USA) with datum WGS84 to enable the return of each individual to their capture site after completion of the experiments. The T_b of active lizards ($N=15$, 8 males and 7 females) was recorded within 15 s of capture by inserting a TP-K01 thermocouple ~5 mm into the cloaca. Micro-environmental temperatures were also recorded at the capture site in order to identify the main heat source used. Substrate temperatures (T_s) were measured by placing the thermocouple (TP-K01) onto the substrate surface, whereas air temperature (T_a) was measured 2 cm above the ground. Thermocouples were connected to a TES 1312A digital thermometer (TES® Electrical Electronic Corp., Taipei, Taiwan, range: –50 to 1300°C, resolution: 0.1/1°C).

We measured operative temperatures (T_e , *sensu* Bakken, 1992) in the field using physical models (Bakken and Angilletta, 2014; Hertz et al., 1993) consisting of a hollow pipe made of three layers of grey propylene with both ends sealed with cork. We placed a temperature logger (Thermochron i-Button DS1921G) in each pipe. Models were calibrated by comparing temperatures of different sizes and colours simultaneously with the cloacal T_b of an adult of *D. leopardinus* exposed to sunshine and shade prior to deployment. The best physical model type was selected according to the best-fit correlation between the model and the T_b of *D. leopardinus* and corresponded to a dull grey PVC pipe, 120 mm length×27 mm diameter (Spearman rank correlation, T_b versus model_{HEATING}, $R=0.99$, $t=13,300.31$, $N=181$, $P<0.001$, $CI=0.99–1.00$; T_b versus model_{COOLING}, $R=0.99$, $t=13,596.7$, $N=183$, $P<0.001$, $CI=0.99–1.00$). We recorded T_e during December of 2018. We deployed 20



Fig. 1. Adult male *Diplolaemus leopardinus* from Angostura valley, Mendoza. Photo credit: A. Laspiur.

physical models in each representative microsite used by the species (exposed rocks, bare soil, beneath shrubs, under rocks) to evaluate the thermal quality of the habitat (Hertz et al., 1993). We programmed the temperature loggers to record every 15 min. We used only the temperature data recorded during the animals' daily period of activity (between 09:00 and 20:00 h) for the analyses.

Laboratory experiments

Animal maintenance

We brought lizards to the laboratory in individual cloth bags. Lizards were housed in the bioterium located at the Instituto Argentino de Investigaciones de las Zonas Áridas (IADIZA, CONICET). Lizards were maintained in standard conditions in an environmental chamber with controlled temperature ($24.0 \pm 1^\circ\text{C}$) and photoperiod (12 h:12 h, light:dark). This temperature was a midpoint between the temperatures of both acclimation treatments. Leopard iguanas were individually located in terraria ($32 \times 45 \times 27$ cm), provided with substratum and rocks collected at the capture site, and had free access to a shelter and basking sites. Water was supplied *ad libitum*, and food (crickets, mealworms and *Blattella* sp. cockroaches) was provided every other day *ad libitum*. Lizards received UV light (Sylvania® Reptistar T8 lamps, Osram Sylvania, Wilmington, MA, USA) 8 h per day. All procedures were performed with the permission of the provincial authority (res. nos. 81/2018 and 96/2018, Dirección de Recursos Naturales Renovables, Mendoza Government) and complied with Argentinian National Law no. 14346 on animal care. The protocols used were approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) of the Universidad Nacional de Cuyo (res. nos. 143/2018).

Preferred body temperatures

We used 21 lizards (10 males and 11 females) in trials to measure preferred body temperature (T_p ; *sensu* Pough and Gans, 1982; Hertz et al., 1993) in a terrarium constructed of fiber-board ($60 \times 120 \times 30$ cm) partitioned into three lanes and partly covered by a ceiling on one end. As previous research suggested that both heliothermic and thigmothermic behaviours have an important role in the thermoregulation of *D. leopardinus* (Laspiur, 2016), we constructed a thermal gradient composed of two heat sources. A 75 W halogen lamp (luminescence equivalent to a 100 W incandescent lamp) was suspended from the ceiling at one end of each lane to generate a heliothermic temperature gradient. The gradient was enhanced by lining one-third of the ceiling and wall surfaces proximate to the lamp with aluminum foil. In addition, to generate the thigmothermic temperature gradient, four thermostat-controlled electric heat coils were connected to four mica sheets (each 30×60 cm), laid on the floor of the terrarium across all lanes, and covered with 5 cm of sand. The first sheet located at the cold end of the terrarium had a 20°C threshold, the second sheet 30°C , the third 40°C and the fourth 50°C . The two heat sources combined to generate a thermal gradient ranging from 23°C to 50°C . We affixed an ultrafine (44 ga) Type T thermocouple to the belly of each lizard using insulation material and surgical tape. The thermocouples were connected to an eight-channel datalogger (Measurement Computing 1.2 kHz Data Acquisition Device, OMEGATC-08 \pm 0.5 $^\circ\text{C}$, Stamford, CT, USA). After 30 min, we recorded T_b at 1 s intervals for 140 min.

After completion of the measurement of thermal preference, we recorded the sex of each individual, and measured their body mass (M_b , 100-g Pesola® micro-line spring scale, ± 1 g), SVL and tail length (digital calliper, 0.01 mm accuracy). Males were identified by an enlargement at the base of the tail caused by the hemipenes,

and if the enlargement was not prominent (i.e. subadults), the hemipenes were everted to confirm sex.

Acclimation treatments

After 1 week of care in the laboratory, 16 individuals were each randomly assigned to one of two acclimation chambers for a 2-week trial. Previous studies have indicated that a 2-week acclimation period is sufficient to elicit phenotypic changes in herpetofauna (Bacigalupe et al., 2018; Barria and Bacigalupe, 2017; Wilhoft and Anderson, 1960). Eight individuals (3 males and 5 females) were housed in the 20°C -acclimation trial ($\pm 2^\circ\text{C}$), which represented the mean operative temperature registered in the field during December 2018, and eight individuals (4 males and 4 females) were housed in the 30°C -acclimation trial ($\pm 2^\circ\text{C}$), which equals the mean preferred temperature of the species, according to our present study. During each trial, we recorded the daily minimum and maximum temperature using a digital thermometer.

Bite endurance and bite force

Bite trials were performed on 20 individuals of *D. leopardinus* (9 males and 11 females). We measured bite endurance and bite force with a piezo-electric isometric force transducer (Kistler® Type 9203 \pm 500N, Kistler Group, Winterthur, Switzerland) fitted with stainless steel plates, connected to an A/D converter that was connected to a computer. The device measures bite force three times per second for 100 s. To avoid damaging the lizard's teeth, the end of each steel plate was covered with leather.

We measured bite endurance and bite force before thermal acclimation treatments (within the first 5 days after capture) and bite force was measured also after acclimation (2 weeks at 20°C or 30°C). For bite endurance and force trials individuals were induced to bite the apparatus three times at each of five cloacal T_b (20 , 24 , 28 , 32 and 34°C) with 20 min rest after each bite and 1 day after each temperature trial. The order of the trials was randomized, and lizards were placed in an environmental chamber at the temperature trial for at least 1 h prior to bite measurements, and cloacal T_b was measured before each bite. We analyzed only front bite position.

To estimate endurance, we used the longest-lasting bite in any one of the three tests at each temperature. We measured endurance as the total time lizards sustained bite force greater than zero (Fig. 2), carefully observing during the trials when lizards were biting the device. For maximum bite force at each temperature, we used the greatest magnitude in any one of the three tests.

Morphometric and maximum bite endurance and force

SVL and M_b were recorded to determine body condition index (BCI). This index was calculated following the methodology proposed by Peig and Green (2009), which consists of calculating the scale mass index (used as an index of body condition) as $\text{BCI} = M_i (\text{SVL}_0 / \text{SVL}_i)^{b_{\text{SMA}}}$, where M_i and SVL_i are the M_b and SVL of the individual, SVL_0 is the arithmetic mean SVL of the population, and b_{SMA} is the standardized major axis slope from the regression of $\ln(\text{mass})$ on $\ln(\text{SVL})$ for the population (Peig and Green, 2009, 2010). The b_{SMA} exponent was calculated using the package *lmodel2* (<https://CRAN.R-project.org/package=lmodel2>) in R (<https://www.r-project.org/>). To evaluate the influence of morphology in bite force and endurance before acclimation treatments, we measured head length (from the tip of the snout to the posterior edge of the parietal bone), head width (the widest part of the head) and jaw length (distance between the jaw articulation to the tip of the lower jaw). All linear measurements were taken with a digital caliper (0.01 mm accuracy).

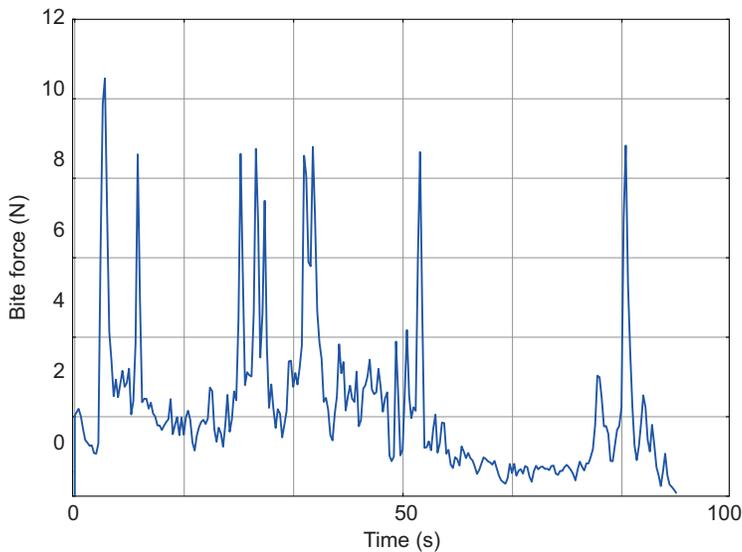


Fig. 2. Bite endurance measurement of a male *D. leopardinus*.
Time (s) for which lizard sustained a bite (N) during the endurance test.

Statistical analyses

The dependence between T_b and micro-environmental temperatures (T_s and T_a) was analyzed using a repeated-measures ANOVA, whereas linear regression was used for the relationship of T_b on T_s and T_a . In order to determine the efficiency of thermoregulation and the preferred body temperature, we used the methodology proposed by Hertz et al. (1993). We calculated the mean preferred body temperature (T_{pmean}) and the mean T_{set} (the interquartile range of T_p) from the T_p obtained from each lizard ($N=21$). The accuracy of T_b was calculated as the mean of the absolute values of the deviations of T_b from the upper or lower bound of T_{set} (individual deviation, d_b). Operative temperature, T_e , was obtained from the mean of the temperatures registered by all the models ($N=20$) during the capture period. To calculate the thermal quality of habitat (d_e) we obtained the mean of the absolute value of the deviations of T_e from the upper or lower bound of T_{set} , large values indicate lower thermal quality (Hertz et al., 1993). Finally, we calculated the index of effectiveness of thermoregulation (E) proposed by Hertz et al. (1993), defined as $E=1-(\text{mean } d_b/\text{mean } d_e)$, which approaches zero when animals do not thermoregulate, approaches one when lizards behave as efficient thermoregulators, and negative values of E occur when lizards avoid thermally high-quality habitats with T_e near or within the range of T_{pmean} (Christian and Weavers, 1996). We compared differences between sex in T_b , T_p , morphometric variables and bite performance using a t -test comparison for parametric data and a Mann–Whitney rank sum test when the assumptions of normality or homogeneity of variance were not fulfilled. We evaluated correlation between morphometric variables using the `cor.test` function in R. We regressed bite endurance and force on head size (head length, head width and jaw length) using the `lm` function of the package `stats v3.6.2` in R.

The influence of T_b on bite endurance and bite force was analyzed within the before-acclimation and the after-acclimation treatments using a generalized mixed linear model (GLMM), with bite endurance or force as the response variable, T_b (explanatory variable), morphometrics and body mass (covariates) as fixed effects, and individual identity as a random variable. We first tested whether individual identity significantly influenced performance across the entire T_b gradient using a χ^2 test. A Gaussian or gamma probability distribution was used depending on the nature of the data. For bite force in the before-acclimation treatment, we used the `glmmadmb` function of the `glmmADMB`

package, with a gamma distribution, because data were not parametric (<http://glmmadmb.r-forge.r-project.org/>; Fournier et al., 2012). We also analyzed the effect of temperature on bite force using a Friedman repeated-measures ANOVA on ranks, and Tukey test as *a posteriori* test. For bite endurance and for force in the after-acclimation treatment, we used the `lme` function of the `nlme` package, with a Gaussian distribution (Bates et al., 2015). Model selection was made using a likelihood ratio test to compare models with different fixed-effect structures throughout the ANOVA function (Zuur et al., 2009). All statistical analyses were performed in R.

RESULTS

Relationship of body temperature (T_b) and preferred body temperature (T_p) with body size and sex

Activity cloacal T_b and T_p did not show significant associations with M_b (linear regression: T_b , $F_{1,15}=4.22$, $P=0.06$; T_p , $F_{1,21}=0.01$, $P=0.92$), SVL (linear regression: T_b , $F_{1,15}=3.37$, $P=0.09$; T_p , $F_{1,21}=0.44$, $P=0.51$) or BCI (linear regression: T_b , $F_{1,15}=0.61$, $P=0.45$; T_p , $F_{1,21}=2.92$, $P=0.1$). Moreover, sexual differences in either T_b or T_p were not found (t -test: T_b , $t_{2,15}=-0.28$, $P=0.78$; T_p , $t_{2,21}=-1.07$, $P=0.30$).

Determination of the main heat source for thermoregulation

Diplolaemus leopardinus showed a mean T_b of $26.69\pm 1.18^\circ\text{C}$ (Table 1, Fig. 3), which was higher than the T_a and did not differ from T_s (repeated-measures ANOVA: $F_{2,15}=49.93$, $P<0.001$, Tukey test: $T_b\times T_a$, $q=12.92$, $P<0.001$; $T_b\times T_s$, $q=1.49$, $P=0.55$). Body temperature depended significantly on both T_s and T_a (linear regression: T_s , $F_{1,15}=22.38$, $P<0.001$, $R^2=0.65$; T_a , $F_{1,15}=11.39$, $P<0.005$, $R^2=0.5$).

Preferred body temperature and effectiveness of thermoregulation (E)

The T_{pmean} was $30.85\pm 0.26^\circ\text{C}$, whereas the mean lower T_{set} was $29.80\pm 1.27^\circ\text{C}$ and the mean upper T_{set} was $31.88\pm 1.40^\circ\text{C}$ (Table 1, Fig. 3). According to the distribution of temperatures recorded in the field, 43% of the records of T_b were included in the T_{set} (Fig. 3), and 57% were below the T_{set} . Mean T_e was $19.42\pm 0.49^\circ\text{C}$, with minimum temperatures of 9°C and maximum temperatures of 44°C during the activity time. The mean d_b was 4.26 ± 0.83 and the mean d_e was 10.31 ± 0.28 . E was 0.57.

Table 1. Snout–vent length (SVL), body mass (M_b), body condition (BCI), activity cloacal body temperature (T_b), preferred temperature (T_{pmean}), and lower and upper set points of T_p (T_{set} lower and T_{set} upper) of *Diplolaemus leopardinus*

Class	SVL (mm)	M_b (g)	BCI	T_b (°C)	T_{pmean} (°C)	T_{set} lower (°C)	T_{set} upper (°C)
Overall	79.69±2.10 (21)	18.70±1.34 (21)	18.52±0.68 (21)	26.69±1.18 (15)	30.85±1.21 (21)	29.80±1.27 (21)	31.88±1.04 (21)
Females	79.99±2.78 (11)	17.97±1.44 (11)	18.68±0.96 (11)	26.28±2.06 (7)	30.59±0.45 (11)	29.63±0.48 (11)	31.54±0.51 (11)
Males	81.87±2.50 (10)	19.98±2.04 (10)	18.52±1.02 (10)	27.00±1.39 (8)	31.54±0.25 (10)	29.99±0.29 (10)	32.26±0.27 (10)

Data are means±s.e.m. (sample size).

Morphometries and maximum bite endurance and force

Males and females did not differ in SVL (t -test: $t_{2,20}=0.49$, $P=0.63$), M_b (t -test: $t_{2,20}=0.82$, $P=0.42$), BCI (t -test: $t_{2,20}=-0.32$, $P=0.75$), head width (Mann–Whitney U -test: $U_{2,20}=45$, $P=0.76$), head length (Mann–Whitney U -test: $U_{2,20}=30$, $P=0.15$), jaw length (t -test: $t_{2,20}=0.52$, $P=0.61$), maximum bite force (t -test: $t_{2,98}=-0.6$, $P=0.56$) or bite endurance (t -test: $t_{2,20}=1.02$, $P=0.32$). Body condition did not influence maximum bite endurance (linear regression: $F_{1,17}=1.31$, $P=0.27$, $R^2=0.07$) or maximum bite force (linear regression: $F_{1,18}=1.15$, $P=0.30$, $R^2=0.06$).

Morphometric variables (head length, head width and jaw length) were correlated (Pearson's correlation: head length and head width: $t_{1,21}=3.93$, $P<0.001$, $r=0.70$; head length and jaw length: $t_{1,21}=4.71$, $P<0.001$, $r=0.74$; head width and jaw length: $t_{1,21}=2.25$, $P<0.05$, $r=0.5$). Maximum bite endurance did not relate to jaw length, head length or head width (Table 2). Maximum bite force was related positively to head length and jaw length, but did not relate to head width (Table 2).

Relationship of body temperature with bite endurance and force before acclimation treatments

None of the explanatory variables were significantly related to bite endurance; the best model was the null model with fixed effects (ANOVA likelihood ratio=6.17; $P=0.19$; Fig. 4A). Table 3 shows the full model with all explanatory variables.

Bite force was negatively influenced by body temperature (Fig. 4B) and by the interaction between body mass and HL. Force was positively influenced by BM and HL (Table 3). In addition, *D. leopardinus* showed differences in bite force among the T_b values analyzed (Friedman repeated-measures ANOVA on ranks: $\chi^2_{4,20}=29.08$, $P<0.001$), with a strong bite magnitude at the lowest body temperatures (Tukey *post hoc* test: 20°C–34°C, $q=5.2$;

20°C–28°C, $q=3$; 24°C–34°C, $q=3.5$; 32°C–34°C, $q=3.1$; all $P<0.05$; Fig. 4B).

Effects of acclimation at different body temperatures on bite force

We found differences in bite force between treatments (Fig. 5): individuals acclimated to 30°C showed greater bite force compared with those acclimated to 20°C (Table 4, Fig. 5).

In the 20°C acclimation treatment, *D. leopardinus* showed differences in bite force among the different T_b analyzed (one-way repeated-measures ANOVA: $F_{4,9}=5.03$, $P<0.05$). The highest T_b studied showed the lowest bite magnitude compared with the rest of body temperatures (Tukey test: 20°C–34°C, $q=5.34$; 24°C–34°C, $q=4.61$; 28°C–34°C, $q=5.34$; all $P<0.05$). In the 30°C acclimation treatment, there were no differences in bite force among the different T_b analyzed (Friedman repeated-measures ANOVA on ranks: $\chi^2_{4,8}=1.72$, $P=0.78$).

DISCUSSION

The leopard iguana inhabits the Altoandina and Patagonica phytogeographic provinces, which are characterized by low mean temperatures, high solar radiation and high daily thermal variation (Corte and Espizua, 1981). In this type of environment, thermoregulatory efficiency is crucial to reduce the costs of thermoregulation such as the energy spent in locomotion or the risks of predation when basking (DeWitt, 1967). *Diplolaemus leopardinus* exhibited a low mean T_b (26.69±1.18°C) compared with other Andean lizards from northern latitudes, such as *Phymaturus antofagastensis* (31.6±2.6°C; Cruz et al., 2009), *P. punae* (29.45±0.48; Ibarguengoytia et al., 2008), *Liolaemus yanalco* (34.16±2.21°C), *L. irregularis* (36.1±1.78°C), *L. multicolor* (36.52±1.33°C) and *L. albiceps* (36.17±1.92°C;

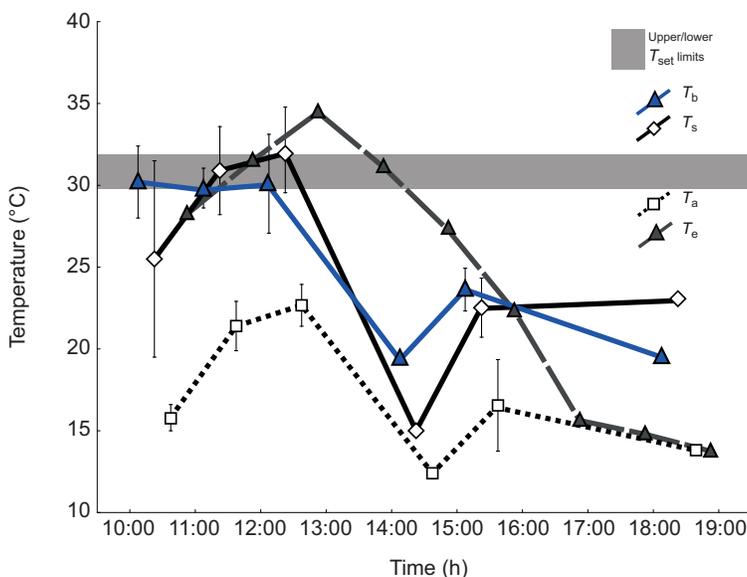


Fig. 3. Temperature variation during daily activity of lizards.

Cloacal body temperature (T_b , blue triangles) measured in the field, substrate temperature (T_s , white diamonds), air temperature (T_a , white squares) and operative temperature (T_e , dark grey triangles) of *D. leopardinus* ($N=15$, 8 males and 7 females). Horizontal grey bar represents the set points of preferred temperature (29.8±1.27 to 31.88±1.04°C). Lines connect the means±s.e.m. of the variables over time.

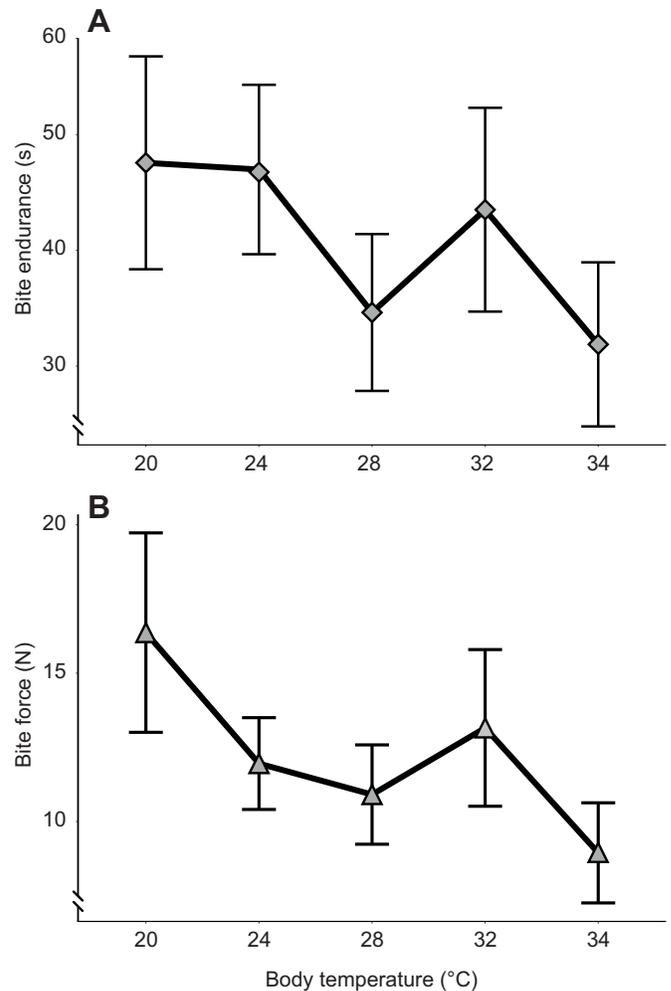
Table 2. Linear regression models for maximum bite endurance and force on morphometric variables of *D. leopardinus* (N=20, 9 males and 11 females)

Model	Variable	Estimate	s.e.	CI		P
				Lower	Upper	
Bite endurance	Head length	-2.45	2.95	-8.67	3.77	0.42
	Head width	2.63	4.14	-6.11	11.37	0.53
	Jaw length	-1.8	2.1	-6.21	2.62	0.4
Bite force	Head length	0.22	0.07	0.08	0.37	<0.05
	Head width	0.16	0.12	-0.96	0.42	0.2
	Jaw length	0.72	0.04	0.11	0.3	<0.001

Valdecantos et al., 2013), *P. palluma* ($31.67 \pm 0.25^\circ\text{C}$; Vicenzi et al., 2017), and *P. eximidilus* ($32.32 \pm 3.13^\circ\text{C}$) and *L. parvus* ($33.74 \pm 2.46^\circ\text{C}$; Gómez Alés et al., 2018). But, the mean T_b was similar to that of other leiosaurids such as *Pristidactylus achalensis* (mean 25.65°C ; Pilustrelli et al., 1987), *P. scapulatus* ($27.00 \pm 1.8^\circ\text{C}$; Villavicencio et al., 2006), *P. torquatus* (22.7°C) and *P. volcanensis* (27.80°C ; Labra, 1995), and *P. valeriae* (26.10°C ; Sufán-Catalán and Núñez, 1993). The dependence of body temperature on T_s shows that *D. leopardinus* is mainly thigmothermic and individuals thermoregulate by shifting among rocks exposed to direct solar radiation, areas shaded by vegetation or rocks, and their shelter. This lizard behaves as a moderate thermoregulator ($E=0.57$), and despite the low thermal quality of their environment ($d_e=10.31 \pm 1.27$), 43% of the lizards were able to attain T_b values in the field that are between the T_{set} values registered in the thermal gradient in the laboratory.

Leopard iguanas were not sexually dimorphic in bite endurance or in bite force. This is in contrast with several species with dimorphism in bite force caused principally by sex differences in body size, head shape and reproductive behaviour (Huyghe et al., 2009; McBrayer and Anderson, 2007). Males have greater M_b and bite force than females in several species such as *Elgaria coerulea* (McBrayer and Anderson, 2007), *Sauromalus ater* (Lappin and Husak, 2005), *Sceloporus clarkii*, *S. undulatus*, *Urosaurus ornatus* and *Cophosaurus texanum* (Meyers and Irschick, 2015), and *Lacerta oxycephala* and *Podarcis melisellensis* (Verwajen et al., 2002). In *D. leopardinus*, the maximum bite force depended on head length and jaw length, as predicted. A larger head results in greater absolute volume of jaw muscle, conferring a bite performance advantage as observed in other lizards in which head size was a good predictor of bite force (Anderson et al., 2008; Herrel et al., 1999, 2001a,b, 2002; Verwajen et al., 2002; Gomes et al., 2020 preprint).

Bite endurance was completely independent of temperature within the range of T_b measured (Fig. 4A); based on these results,

**Fig. 4. Mean \pm s.d. bite performance in relation to temperature.** (A) Bite endurance (s) and (B) bite force (N) over the five body temperatures ($^\circ\text{C}$) tested in *D. leopardinus* (N=20, 9 males and 11 females) before thermal acclimation treatments.

we can hypothesize that the muscle fibers used for sustaining tension are less sensitive to temperature than those involved in the generation of force. Whereas great force requires fast-twitch muscle fibers, which are more easily exhausted, endurance needs slow-twitch muscle fibers, which are less easily fatigued albeit incapable of great force (Herrel et al., 1999). Previous studies, such as Gomes et al. (2020 preprint), have evaluated this novel trait in *Podarcis bocagei* as the time at which the bite force curve crossed at half the

Table 3. Generalized linear mixed models of bite endurance and force on body temperature and body mass with morphometries as covariables of *D. leopardinus* (N=20, 9 males and 11 females)

Model	Variable	Estimate	s.e.	CI		P
				Lower	Upper	
Bite endurance	Intercept	101.5	26.49	48.72	154.30	<0.05
	Body temperature	-0.92	0.59	-2.10	0.26	0.12
	Body mass	-1.80	1.04	-3.99	0.39	0.10
Bite force	Intercept	-3.18	1.86	-6.82	0.46	0.08
	Body temperature	-0.03	0.01	-0.05	-0.01	<0.05
	Body mass	0.26	0.09	0.09	0.44	<0.05
	Head length	0.23	0.08	0.07	0.38	<0.05
	Body mass:Head length	-0.01	0.003	-0.02	-0.002	<0.05

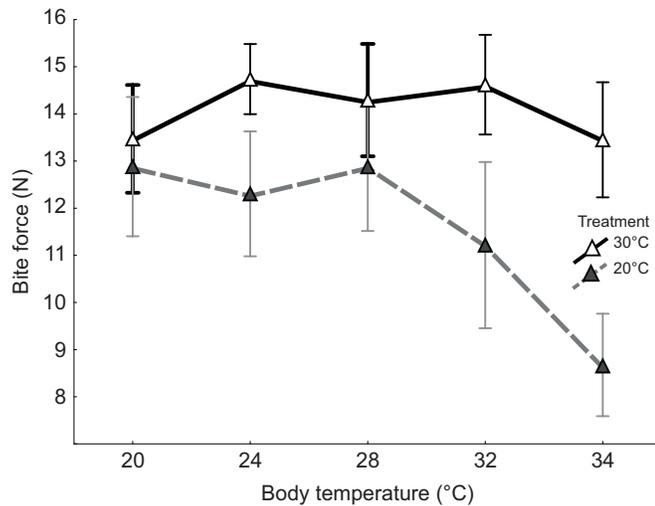


Fig. 5. Mean \pm s.d. bite force in relation to temperature. Bite force (N) versus body temperature ($^{\circ}$ C) in *D. leopardinus* acclimated to 30 $^{\circ}$ C (N=8) and 20 $^{\circ}$ C (N=8).

maximal value. Those authors found that, in contrast to bite force, bite endurance is not influenced by sex, and is probably related to muscle architecture, which shows few differences between males and females in *Podarcis* species (Gomes et al., 2020 preprint). Accordingly, we did not find differences between sexes in endurance; however, we measured bite duration differently than Gomes et al. (2020 preprint), because we considered the total time that lizards maintained a sustained full-mouth grip (Fig. 2). *Diplolaemus leopardinus* showed a bite pattern in which shorter peaks of bite magnitude alternated with periods of sustained bite. This bite pattern is congruent with feeding behavior, where longer periods of prey holding could alternate with short mash chewing movements (N. Vicenzi, unpublished observations). Moreover, this bite pattern is consistent with mating behavior in leiosaurid lizards. During courtship and mating, the male subjugates the female by sustaining neck-bite hold for more than 1 h (A. Laspiur, unpublished data). The male assumes a dominant position on the female dorsum with a sustained neck-bite hold, then strongly grasps the skin of the neck so as to stretch the neck and lift the stiff body of the female, and bring the cloaca closer to that of the male to facilitate the introduction of the hemipenis (A. Laspiur, unpublished data). This type of behavior was observed in several other species of lizards, such as *P. bocagei* (Galán, 1997), *Tropidurus spinulosus* (Pelegrin, 2019) and *Phymaturus* cf. *palluma* (Eisenberg and Werning, 2012).

Table 4. Generalized linear mixed model between bite force and body temperature and acclimation treatments (treatment at 20 $^{\circ}$ C and treatment at 30 $^{\circ}$ C) of *D. leopardinus* (N=16)

Variable	Estimate	s.e.	CI		P
			Lower	Upper	
Intercept	-19.26	6.79	-32.84	-5.68	<0.05
20 $^{\circ}$ C treatment	7.79	3.03	1.24	14.34	<0.05
Body temperature \times 30 $^{\circ}$ C treatment	0.005	0.07	-0.14	0.15	0.94
Body temperature \times 20 $^{\circ}$ C treatment	-0.29	0.1	-0.50	-0.08	<0.05
Head length	1.24	0.24	0.72	1.76	<0.05

Mean maximum bite force, before acclimation treatments (recorded at 20 $^{\circ}$ C T_b), dropped by more than half at 34 $^{\circ}$ C T_b (Fig. 4B). In contrast, previous studies have shown that muscle force decreases significantly below 25 $^{\circ}$ C (Herrel et al., 2007; Marsh and Bennett, 1986); in *D. leopardinus*, the better temperature for maximum bite force performance was 20 $^{\circ}$ C, lower than the mean T_b of activity (26.69 \pm 1.18 $^{\circ}$ C) and lower than the T_{pmean} of the species (30.85 \pm 0.26 $^{\circ}$ C). Similar results were found in *B. pumilum*, which has an optimal bite temperature of 23.7 \pm 1.0 $^{\circ}$ C and a T_{pmean} of 29.3 \pm 1.3 $^{\circ}$ C (Segall et al., 2013). The better temperature of 20 $^{\circ}$ C was similar to the minimum T_b of activity registered in the field (19.50 $^{\circ}$ C) and the mean T_e obtained at the study site (19.42 \pm 0.49 $^{\circ}$ C). These results suggest that if lizards are cold, they would still be able to feed and defend themselves, and they may prefer these low temperatures even though they have the potential to attain higher body temperatures by thermoregulation. The maximum bite force recorded at low T_b in the leopard iguana could be related to the 'fight versus flight' behavioural response observed in several species of lizard (Herrel et al., 2007; Hertz et al., 1982). According to the theory on this behavioral response, at higher T_b lizards escape quickly from potential predators, but at lower T_b they do not run but stand their ground and attack aggressively (Hertz et al., 1982). Greater bite force at lower T_b by leopard iguanas is thus consistent with this theory. Several authors suggest that behavioural functions can generate evolutionary changes in morphology, physiology and ecology (e.g. Mayr, 1963; Wyles et al., 1983), and the 'fight versus flight' behaviour of *D. leopardinus* reinforces this hypothesis.

Bite force proved to be a plastic phenotypic trait when individuals were exposed to short-term acclimation treatments (Fig. 5). Phenotypic plasticity is expected in species that inhabit highly variable or unpredictable environments, such as the one in this study, as long as reliable cues exist to prompt beneficial plastic responses and the costs of plasticity are relatively low (Angilletta, 2009; Ghalambor et al., 2007; Marais and Chown, 2008). Leopard iguanas showed plasticity in both degree and pattern of the thermal function between T_b and bite force when acclimated to a constant temperature (Fig. 5). In animals acclimated at 30 $^{\circ}$ C, we observed an increase in performance at higher temperatures, as the 'hotter is better' hypothesis predicts, but also a different pattern as bite force was almost constant across all T_b measurements (Fig. 5). This means that lizards exhibit changes in the slope value of the thermal reaction function and in its pattern (Pigliucci, 2001). Greater bite force implies the ability to capture larger prey and to reduce the time needed to handle and subdue prey, improving foraging efficiency (Herrel et al., 2001b, 1996). At higher acclimation temperatures, an increase in metabolic rate and, consequently, an increase in food requirements are expected (Dillon et al., 2010). Thus the greater bite force observed in the 30 $^{\circ}$ C treatment could have ecological relevance because it improves prey quality and reduces handling cost.

Usually, studies about the thermal sensitivity of performance traits are based on physiological variables considered to be highly temperature dependent, such as locomotion speed or endurance, and not on traits typically considered less temperature dependent, such as bite and tail force (but see Diele-Viegas and Rocha, 2018; Segall et al., 2013). In this study, we focused on bite endurance and force, traits considered to have low temperature dependence and hence less affected by the increase in temperatures in a global warming scenario. However, we found that for *D. leopardinus*, temperature exerts a significant influence on bite force, and moreover, that bite force is a trait that showed thermal plasticity in short-term acclimations. Our results suggest that, at least in this trait, lizards are able to maintain and even improve bite performance with the

projected rising temperatures. However, we also must consider that, in the Central Andes, the predictions for future climate change include more than an increase in mean temperature; they also include an increase in the frequency of extreme events such as drought and heat waves (Barros et al., 2014). Our study of acclimation was carried out with controlled and constant temperatures and with water *ad libitum*. Thus, a fuller understanding of lizard responses to global warming would require studies incorporating temperature variation and other sources of stress such as drought.

In the leopard iguana, T_{pmean} is significantly higher than maximum body temperature for bite force in lizards before acclimation, probably related to the fact that different physiological processes are taking place, influencing the thermal coadaptation between T_p and optimum temperature (Angilletta, 2009). Such differences in thermal optimum of other traits has been observed in several species, such as *Liolaemus sarmientoi*, in which T_{pmean} (34.43°C) was similar to optimum temperatures for long runs (34°C), but much higher than for sprint runs (27°C; Fernández et al., 2011). As with bite force, sprint runs are mostly related to defensive behaviors (Cabezas-Cartes et al., 2014). Present results indicate that this population of *D. leopardinus* shows phenotypic plasticity in bite force in laboratory conditions; thus lizards may have the plasticity to acclimate to short-term changes in environmental temperatures, and they may be able to adapt to a more gradual change, at least in bite force. However, according to our results, it seems possible that at higher temperatures (as exemplified by lizards acclimated to 30°C), individuals may lose their thermal dependence in bite force in response to thermal changes (Fig. 4B). This loss could produce a decoupling of the 'fight or flight' behavioral strategy and a significant increase in maintenance cost for the individuals. To sustain a maximal performance across different body temperatures, in both thermally dependent traits, would represent a significant increase in maintenance costs for the individuals, which could compromise the energy balance with negative implications for the persistence of the species.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: N.V., P.L.S., N.R.I.; Methodology: N.V., A.L., P.L.S., R.M., J.K., N.R.I.; Software: A.L.; Formal analysis: N.V., A.L., N.R.I.; Investigation: N.V., A.L., J.K., N.R.I.; Resources: P.L.S., R.M., N.R.I.; Data curation: N.V., A.L., P.L.S., R.M., J.K., N.R.I.; Writing - original draft: N.V., A.L., N.R.I.; Writing - review & editing: N.V., A.L., P.L.S., R.M., J.K., N.R.I.; Visualization: N.V., A.L., P.L.S., R.M., J.K.; Supervision: P.L.S., N.R.I.; Project administration: N.V.; Funding acquisition: N.V., P.L.S., N.R.I.

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References

Adolph, S. C. and Porter, W. P. (1993). Temperature, activity, and lizard life histories. *Am. Nat.* **142**, 273-295. doi:10.1086/285538

Anderson, R. A., Mcbrayer, L. D. and Herrel, A. (2008). Bite force in vertebrates: opportunities and caveats for use of a nonpareil whole-animal performance measure. *Biol. J. Linn. Soc.* **93**, 709-720. doi:10.1111/j.1095-8312.2007.00905.x

Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. New York: Oxford, University Press.

Angilletta, M. J., Niewiarowski, P. H. and Navas, C. A. (2002). The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* **27**, 249-268. doi:10.1016/S0306-4565(01)00094-8

Angilletta, M. J., Jr, Bennett, A. F., Guderley, H., Navas, C. A., Seebacher, F. and Wilson, R. S. (2006). Coadaptation: A unifying principle in evolutionary thermal biology. *Physiol. Biochem. Zool.* **79**, 282-294. doi:10.1086/499990

Bacigalupe, L. D., Gaitán-Espitia, J. D., Barria, A. M., Gonzalez-Mendez, A., Ruiz-Aravena, M., Trinder, M. and Sinervo, B. (2018). Natural selection on plasticity of thermal traits in a highly seasonal environment. *Evol. Appl.* **11**, 2004-2013. doi:10.1111/eva.12702

Bakken, G. S. (1992). Measurement and application of standard and operative temperatures in ecology. *Am. Zool.* **32**, 194-216. doi:10.1093/icb/32.2.194

Bakken, G. S. and Angilletta, M. J. (2014). How to avoid errors when quantifying thermal environments. *Funct. Ecol.* **28**, 96-107. doi:10.1111/1365-2435.12149

Barria, A. M. and Bacigalupe, L. D. (2017). Intraspecific geographic variation in thermal limits and acclimatory capacity in a wide distributed endemic frog. *J. Therm. Biol.* **69**, 254-260. doi:10.1016/j.jtherbio.2017.08.010

Barros, V. R., Boninsegna, J. A., Camilloni, I. A., Chidiak, M., Magrín, G. O. and Rusticucci, M. (2014). Climate change in Argentina: trends, projections, impacts and adaptation. *Wiley Interdiscip. Rev. Clim. Chang.* **6**, 151-169. doi:10.1002/wcc.316

Bates, D., Mäechler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed effects models using lme4. *J. Stat. Softw.* **67**, 1-48. doi:10.18637/jss.v067.i01

Bennett, A. F. (1985). Temperature and muscle. *J. Exp. Biol.* **115**, 333-344.

Bestion, E., Teyssier, A., Richard, M., Clobert, J. and Cote, J. (2015). Live fast, die young: experimental evidence of population extinction risk due to climate change. *PLoS Biol.* **13**, e1002281. doi:10.1371/journal.pbio.1002281

Black, I. R. G., Berman, J. M., Cadena, V. C. and Tattersall, G. J. (2019). Behavioral thermoregulation in lizards. Strategies for achieving preferred temperature. In *Behavior of Lizards. Evolutionary and Mechanistic Perspectives* (ed. V. L. Bels and A. P. Russell), pp. 13-46. CRC Press.

Cabezas-Cartes, F., Kubisch, E. L. and Ibagüengoytia, N. R. (2014). Consequences of volcanic ash deposition on the locomotor performance of the *Phymaturus spectabilis* lizard from Patagonia, Argentina. *J. Exp. Zool. Part A Ecol. Genet. Physiol.* **321**, 164-172. doi:10.1002/jez.1846

Cameron, S. F., Wheatley, R. and Wilson, R. S. (2018). Sex-specific thermal sensitivities of performance and activity in the Asian house gecko, *Hemidactylus frenatus*. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **188**, 635-647. doi:10.1007/s00360-018-1149-2

Cei, J. M. (1986). *Reptiles del centro, centro-oeste y sur de la Argentina. Herpetofauna de las zonas áridas y semiáridas*, p. 527. Torino: Museo Regionale di Scienze Naturali, Monografia IV.

Christian, K. A. and Weavers, B. W. (1996). Thermoregulation of monitor lizards in Australia: an evaluation of methods in thermal biology. *Ecol. Monographs* **66**, 139-157. doi:10.2307/2963472

Corte, A. E. and Espizua, L. E. (1981). Clima. In *Inventario de Glaciares de la Cuenca del Río Mendoza* (ed. IANIGLA- CONICET), pp. 9-10. Mendoza, Argentina: Litografía e Imprenta Farras, Mendoza.

Cruz, F. B., Belver, L., Acosta, J. C., Villavicencio, H. J., Blanco, G. and Cánovas, M. G. (2009). Thermal biology of *Phymaturus* lizards: evolutionary constraints or lack of environmental variation? *Zoology* **112**, 425-432. doi:10.1016/j.zool.2009.03.004

Dawson, T. P., Jackson, S. T., House, J. I., Prentice, I. C. and Mace, G. M. (2011). Beyond predictions: biodiversity conservation in a changing climate. *Science* **332**, 53-58. doi:10.1126/science.1200303

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C. and Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. USA* **105**, 6668-6672. doi:10.1073/pnas.0709472105

DeWitt, C. B. (1967). Precision of thermoregulation and its relation to environmental factors in desert iguana, *Dipsosaurus dorsalis*. *Physiol. Zool.* **40**, 49-66. doi:10.1086/physzool.40.1.30152438

Diele-Viegas, L. M. and Rocha, C. F. D. (2018). Unraveling the influences of climate change in Lepidosauria (Reptilia). *J. Therm. Biol.* **78**, 401-414. doi:10.1016/j.jtherbio.2018.11.005

Dillon, M. E., Wang, G. and Huey, R. B. (2010). Global metabolic impacts of recent climate warming. *Nature* **467**, 704-706. doi:10.1038/nature09407

Donoso-Barros, R. (1966). *Reptiles de Chile*. Santiago: Universidad de Chile.

Eisenberg, T. and Werning, H. (2012). *Phymaturus cf. palluma* in captivity: observations on its reproduction and biology. *Salamandra* **48**, 198-206.

Erickson, G. M., Lappin, A. K., Parker, T. and Vliet, K. A. (2004). Comparison of bite-force performance between long-term captive and wild American alligators (*Alligator mississippiensis*). *J. Zool.* **262**, 21-28. doi:10.1017/S0952836903004400

Fernández, J. B., Smith, J., Scolaro, A. and Ibagüengoytia, 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. doi:10.1016/j.jtherbio.2010.09.006

- Fournier, D. A., Skaug, H. J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M., Nielsen, A. and Sibert, J. (2012). AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex non linear models. *Optim. Methods Softw.* **27**, 233-249. doi:10.1080/10556788.2011.597854
- Frost, D. R., Etheridge, R., Janies, D. and Titus, T. A. (2001). Total evidence, sequence alignment, evolution of polychrotid lizards, and a reclassification of the Iguania (Squamata: Iguania). *Am. Mus. Novit.* **3343**, 1-38. doi:10.1206/0003-0082(2001)343<0001:TESAEO>2.0.CO;2
- Galán, P. (1997). Reproductive ecology of the lacertid lizard *Podarcis bocagei*. *Ecography* **20**, 197-209. doi:10.1111/j.1600-0587.1997.tb00362.x
- Ghalambor, C. K., McKay, J. K., Carroll, S. P. and Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* **21**, 394-407. doi:10.1111/j.1365-2435.2007.01283.x
- Gomes, V., Herrel, A., Carretero, M. A. and Kaliontzopoulou, A. (2020). New insights into bite performance: morphological trade-offs underlying the duration and magnitude of bite force. *Physiol. Biochem. Zool.* **93**, 175-184. doi:10.1086/708248
- Gómez Alés, R., Acosta, J. C., Astudillo, V., Córdoba, M., Blanco, G. M. and Miles, D. (2018). Effect of temperature on the locomotor performance of species in a lizard assemblage in the Puna region of Argentina. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **188**, 977-990. doi:10.1007/s00360-018-1185-y
- Halliday, W. D. and Blouin-Demers, G. (2017). A test of the thermal coadaptation hypothesis with ultimate measures of fitness in flour beetles. *J. Therm. Biol.* **69**, 206-212. doi:10.1016/j.jtherbio.2017.07.017
- Herrel, A. and O'Reilly, J. C. (2006). Ontogenetic scaling of bite force in lizards and turtles. *Physiol. Biochem. Zool.* **79**, 31-42. doi:10.1086/498193
- Herrel, A., Aerts, P., Fret, J. and De Vree, F. (1999). Morphology of the feeding system in agamid lizards: ecological correlates. *Anat. Rec.* **254**, 496-507. doi:10.1002/(SICI)1097-0185(19990401)254:4<496::AID-AR5>3.0.CO;2-Q
- Herrel, A., Grauw, E. D. D. E. and Lemos-espinal, J. A. (2001a). Head shape and bite performance in xenosaurid lizards. *J. Exp. Zool.* **107**, 101-107. doi:10.1002/jez.1039
- Herrel, A., Van Damme, R., Vanhooydonck, B. and De Vree, F. (2001b). The implications of bite performance for diet in two species of lacertid lizards. *Can. J. Zool.* **670**, 662-670. doi:10.1139/z01-031
- Herrel, A., Meyers, J. J. and Vanhooydonck, B. (2002). Relations between microhabitat use and limb shape in phrynosomatid lizards. *Biol. J. Linn. Soc.* **77**, 149-163. doi:10.1046/j.1095-8312.2002.00101.x
- Herrel, A., James, R. S. and Van Damme, R. (2007). Fight versus flight: physiological basis for temperature-dependent behavioral shifts in lizards. *J. Exp. Biol.* **210**, 1762-1767. doi:10.1242/jeb.003426
- Herrel, A., Van Damme, R. and DeVree, F. (1996). Sexual dimorphism of head size in *Podarcis hispanica*: testing the dietary divergence hypothesis by bite force analysis. *Neth. J. Zool.* **46**, 253-262.
- Hertz, P. E., Huey, R. B. and Nevo, E. (1982). Fight versus flight: body temperature influences defensive responses of lizards. *Anim. Behav.* **30**, 676-679. doi:10.1016/S0003-3472(82)80137-1
- Hertz, P. E., Huey, R. B. and Stevenson, R. D. (1993). Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *Am. Nat.* **142**, 796-818. doi:10.1086/285573
- Huey, R. B. and Bennett, A. F. (1987). Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. *Evolution* **41**, 1098-1115. doi:10.1111/j.1558-5646.1987.tb05879.x
- Huey, R. B. and Berrigan, D. A. (1996). Testing evolutionary hypotheses of acclimation. In *Animals and Temperature: Phenotypic and Evolutionary Adaptation* (ed. I. A. Johnston and A. F. Bennett). pp. 205-237. Society for Experimental Biology Seminar Series. Cambridge: Cambridge University Press.
- Huey, R. B. and Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* **4**, 131-135. doi:10.1016/0169-5347(89)90211-5
- Husak, J. F., Lappin, A. K., Fox, S. F. and Lemos-Espinal, J. A. (2006). Bite-force performance predicts dominance in male vulnerable collared lizards (*Crotaphytus antiquus*). *Copeia* **2006**, 301-306. doi:10.1643/0045-8511(2006)6[301:BPPDIM]2.0.CO;2
- Huyghe, K., Vanhooydonck, B., Scheers, H., Molina-Borja, M. and Van Damme, R. (2005). Morphology, performance and fighting capacity in male lizards, *Gallotia galloti*. *Funct. Ecol.* **19**, 800-807. doi:10.1111/j.1365-2435.2005.01038.x
- Huyghe, K., Herrel, A., Adriaens, D., Tadic, Z. and Van Damme, R. (2009). It is all in the head: morphological basis for differences in bite force among colour morphs of the Dalmatian wall lizard. *Biol. J. Linn. Soc.* **96**, 13-22. doi:10.1111/j.1095-8312.2008.01103.x
- Ibargüengoytia, N. R., Bird, P. S., Uzal, F. A. and Cipolla, A. L. (2004). Oral microbiota of patagonian lizards of genus *Diplolaemus* (Leiosauridae): fable to facts. *Cuad. Herpetol* **18**, 37-41.
- Ibargüengoytia, N. R., Acosta, J. C., Boretto, J. M., Villavicencio, H. J., Marinero, J. and 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. doi:10.1016/j.jaridenv.2008.03.018
- IPCC (2018). *Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty.* Geneva: World Meteorological Organization.
- Kubisch, E., Piantoni, C., Williams, J., Sclaro, A., Navas, C. A. and Ibargüengoytia, N. R. (2012). Do higher temperatures increase growth in the nocturnal gecko *Homonota darwini* (Gekkota: Phyllodactylidae)? a skeletochronological assessment analyzed at temporal and geographic scales. *J. Herpetol.* **46**, 587-595. doi:10.1670/10-277
- Labra, A. (1995). Thermoregulation in *Pristidactylus* lizards (Polycridae): effects of group size. *J. Herpetol.* **29**, 260-264. doi:10.2307/1564563
- Lailvaux, S. P., Herrel, A., Vanhooydonck, B., Meyers, J. J. and Irschick, D. J. (2004). Performance capacity, fighting tactics and the evolution of life-stage male morphs in the green anole lizard (*Anolis carolinensis*). *Proc. Biol. Sci.* **271**, 2501-2508. doi:10.1098/rspb.2004.2891
- Lappin, A. K. and Husak, J. F. (2005). Weapon performance, not size, determines mating success and potential reproductive output in the collared lizard (*Crotaphytus collaris*). *Am. Nat.* **166**, 426-436. doi:10.1086/432564
- Laspiur, A. (2016). Sistemática de las lagartijas de la Subfamilia Leiosaurinae: Filogenia y biogeografía histórica. *PhD thesis*, Universidad Nacional de Río Cuarto, Córdoba.
- Laspiur, A., Sanabria, E. and Acosta, J. C. (2007). Primeros datos sobre vocalización en *Leiosaurus catamarcensis* (Koslowky, 1898) y *Pristidactylus scapularis* Burmeister, 1861 (Iguania, Leiosauridae) de San Juan, Argentina. *Rev. Peru. Biol.* **14**, 217-220. doi:10.15381/rpb.v14i2.1740
- Leroi, A. M., Bennett, A. F. and Lenski, R. E. (1994). Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proc. Natl. Acad. Sci. USA* **91**, 1917-1921. doi:10.1073/pnas.91.5.1917
- Llewelyn, J., Macdonald, S. L., Moritz, C., Martins, F., Hatcher, A. and Phillips, B. L. (2018). Adjusting to climate: acclimation, adaptation, and developmental plasticity in physiological traits of a tropical rainforest lizard. *Integr. Zool.* **13**, 411-427. doi:10.1111/1749-4877.12309
- Marais, E. and Chown, S. L. (2008). Beneficial acclimation and the Bogert effect. *Ecol. Lett.* **11**, 1027-1036. doi:10.1111/j.1461-0248.2008.01213.x
- Marsh, R. L. and Bennett, A. F. (1986). Thermal dependence of contractile muscle from the lizard *Sceloporus occidentalis* with comments on methods for fitting and comparing force-velocity curves. *J. Exp. Biol.* **126**, 63-77.
- Mayr, E. (1963). *Animal Species and Evolution*. Cambridge: Harvard University Press.
- McBrayer, L. D. and Anderson, R. A. (2007). Sexual size dimorphisms and bite force in the northern alligator lizard, *Elgaria coerulea*. *J. Herpetol.* **41**, 554-559. doi:10.1670/07-045.1
- Meyers, J. J. and Irschick, D. J. (2015). Does whole-organism performance constrain resource use? A community test with desert lizards. *Biol. J. Linn. Soc.* **115**, 859-868. doi:10.1111/bj.12537
- Peig, J. and Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* **118**, 1883-1891. doi:10.1111/j.1600-0706.2009.17643.x
- Peig, J. and Green, A. J. (2010). The paradigm of body condition: a critical reappraisal of current methods based on mass and length. *Funct. Ecol.* **24**, 1323-1332. doi:10.1111/j.1365-2435.2010.01751.x
- Pelegrin, N. (2019). Reproductive behavior of *Tropidurus spinulosus* (Squamata: Tropiduridae) in captivity. *Phyllomedusa* **18**, 123-126. doi:10.11606/issn.2316-9079.v18i1p123-126
- Pigliucci, M. (2001). *Phenotypic Plasticity: Beyond Nature and Nurture*. Baltimore: Johns Hopkins University Press.
- Pilustrelli, C., Tada, I. E. and Martori, R. (1987). Termorregulación de *Pristidactylus achalensis* (Sauria: iguanidae). *Boletín de la Asociación Herpetológica Argentina* **3**, 3. <http://sedici.unlp.edu.ar/handle/10915/6159>
- Pough, F. H. and Gans, C. (1982). Biology of the Reptilia. In *The Vocabulary of Reptilian Thermoregulation* (ed. C. Gans and F. H. Pough), pp. 17-24. New York: Academic Press.
- Schulte, P. M., Healy, T. M. and Fanguie, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr. Comp. Biol.* **51**, 691-702. doi:10.1093/icb/ict097
- Segall, M., Tolley, K., Vanhooydonck, B., Measey, J. and Herrel, A. (2013). Impact of temperature on performance in two species of South African dwarf chameleon, *Bradypodion pumilum* and *B. occidentale*. *J. Exp. Biol.* **286**, 3828-3836. doi:10.1242/jeb.092353
- Sileo, N., Trombotto, D. and Dapeña, C. (2015). Estudios preliminares del agua, nieve y hielo en la cuenca del río Vallecitos, Mendoza, Argentina. *Acta Geológica Lilloana* **27**, 130-145.
- Sinervo, B., Méndez-de-la-Cruz, F., Miles, D. B., Heulin, B., Bastiaans, E., Villagrán-Santa Cruz, M., Lara-Resendiz, R., Martínez Méndez, N., Calderón-Espinosa, M. L., Meza-Lázaro, R. N. et al. (2010). Erosion of lizard diversity by climate change and altered thermal niches. *Science* **328**, 894-899. doi:10.1126/science.1184695
- Sinervo, B., Miles, D. B., Yayong, W. U., Méndez-De La Cruz, F. R., Kirchoff, S. and Yin, Q. I. (2018). Climate change, thermal niches, extinction risk and

- maternal-effect rescue of toad-headed lizards, *Phrynocephalus*, in thermal extremes of the Arabian Peninsula to the Qinghai–Tibetan Plateau. *Integr. Zool.* **13**, 450–470. doi:10.1111/1749-4877.12315
- Sufán-Catalán, J. and Nuñez, H.** (1993). Estudios autecológicos en *Pristidactylus cf. valeriae* (Squamata, Polychridae) de Chile central. *Bol. Mus. Nac. Hist. Nat* **44**, 115–130.
- Valdecantos, S., Martínez, V., Lobo, F. and Cruz, F. B.** (2013). Thermal biology of *Liolaemus* lizards from the high Andes: being efficient despite adversity. *J. Therm. Biol.* **38**, 126–134. doi:10.1016/j.jtherbio.2012.12.002
- Van Devender, T. R.** (1977). Observations on the argentine iguanid lizard *Leiosaurus bellii* Duméril and Bibron (Reptilia, Lacertia; Iguanidae). *J. Herpetol.* **11**, 238–241. doi:10.2307/1563156
- Verwajen, D., Van Damme, R. and Herrel, A.** (2002). Relationships between head size, bite force, prey handling efficiency and diet in two sympatric lacertid lizards. *Funct. Ecol.* **16**, 842–850. doi:10.1046/j.1365-2435.2002.00696.x
- Vicenzi, N., Corbalán, V., Miles, D., Sinervo, B. and Ibagüengoytia, N.** (2017). Range increment or range detriment? Predicting potential changes in distribution caused by climate change for the endemic high-Andean lizard *Phymaturus palluma*. *Biol. Conserv.* **206**, 151–160. doi:10.1016/j.biocon.2016.12.030
- Victoriano, P. F., Coronado, T. M. and Ortiz, J. C.** (2010). A multivariate analysis of taxonomic limits in *Diplolaemus* Bell 1843. *Gayana (Concepción)* **74**, 23–36. doi:10.4067/S0717-65382010000100006
- Villavicencio, H. J., Acosta, J. C. and Marinero, J. A.** (2006). *Pristidactylus scapulatus* (NCN) body temperature. *Herpetol. Rev.* **37**, 471.
- Wilhoft, D. and Anderson, J.** (1960). Effect of acclimation on the preferred body temperature of the lizard, *Sceloporus occidentalis*. *Science* **131**, 610–611. doi:10.1126/science.131.3400.610
- Wilson, R. S. and Franklin, C. E.** (2002). Testing the beneficial acclimation hypothesis. *Trends Ecol. Evol.* **17**, 66–70. doi:10.1016/S0169-5347(01)02384-9
- Wyles, J. S., Kunkel, J. G. and Wilson, A. C.** (1983). Birds, behavior, and anatomical evolution. *Proc. Natl. Acad. Sci. USA* **80**, 4394–4397. doi:10.1073/pnas.80.14.4394
- Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. and Smith, G. M.** (2009). *Mixed Effects Models and Extensions in Ecology with R*. New York: Springer.