RESEARCH ARTICLE

Could plasticity mediate highlands lizards' resilience to climate change? A case study of the leopard iguana (*Diplolaemus leopardinus*) in Central Andes of Argentina

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ABSTRACT

The predicted rise of global temperatures is of major concern for ectotherms because of its direct impact on their behavior and physiology. As physiological performance mediates a species' resilience to warming exposure, physiological plasticity could greatly reduce the susceptibility to climate change. We studied the degree to which *Diplolaemus leopardinus* lizards are able to adjust behavioral and physiological traits in response to short periods of temperature change. We used a split cross design to measure the acclimation response of preferred body temperature (T_p), and the thermal performance curve of resting metabolic rate (RMR) and evaporative water loss (EWL). Our results showed that plasticity differs among traits: whereas T_p and EWL showed lower values in warm conditions, the body temperature at which RMR was highest increased. Moreover, RMR was affected by thermal history, showing a large increase in response to cold exposure in the group initially acclimated to warm temperatures. The reduction of EWL and the increase in optimal temperature will give lizards the potential to partially mitigate the impact of rising temperatures in the energy cost and water balance. However, the decrease in T_p and the sensitivity to the warm thermal history of RMR could be detrimental to the energy net gain, increasing the species' vulnerability, especially considering the increase of heat waves predicted for the next 50 years. The integration of acclimation responses in behavioral and physiological traits provides a better understanding of the range of possible responses of lizards to cope with the upcoming climatic and environmental modifications expected as a result of climate change.

KEY WORDS: Metabolism, Phenotypic plasticity, Reptiles, Thermal preference, Water loss

INTRODUCTION

Climate change represents one of the most important anthropogenic threats affecting the global biota. According to the IPCC (2013), heat waves and drought will intensify in both duration and frequency in the coming years, increasing the already high thermal stress and

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water deficiency experienced by organisms. The rising temperatures predicted are of major concern for ectotherms because they directly impact the metabolic rate, water balance, digestion, growth, reproduction, activity, survival and therefore the fitness of this group (Black et al., 2019). As physiological performance mediates a species' resilience to warming exposure (Bozinovic et al., 2011), physiological plasticity could greatly reduce the susceptibility to climate change (Stillman, 2003; Seebacher et al., 2015), especially if the potential to carry out behavioral adjustments is reduced (e.g. behavioral thermoregulation; Gunderson and Stillman, 2015).

Phenotypic plasticity refers to the ability of a single genotype to produce different phenotypes when exposed to different environmental conditions (Pigliucci, 2001). Thermal acclimation represents a kind of phenotypic plasticity that implies reversible changes in biological traits as a result of an organism's exposure to variation in thermal conditions (Schulte et al., 2011). One approach to measure different plastic responses to the thermal environment is through the quantification of changes in the magnitude and pattern of thermal performance curves (TPCs), which relate instantaneous physiological rates and individuals' body temperature (Huey and Stevenson, 1979; Sinclair et al., 2016). Because the magnitude and pattern of plasticity can be different among traits (Murren et al., 2014), an integrated study of acclimation throughout multiple physiological traits is crucial to reveal whether these adjustments are beneficial for the whole organism and, therefore, would increase a species' resilience to global warming (Pigliucci, 2001; Bozinovic et al., 2011; Magozzi and Calosi, 2015).

Preferred body temperature (T_p), resting metabolic rate (RMR) and evaporative water loss (EWL) are tightly related traits usually sensitive to changes in the thermal conditions of the environment, and shifts in one could impact any of the others (Basson and Clusella-Trullas, 2015). T_p is the range of body temperatures (T_b) freely selected by an organism in a thermal gradient (Licht et al., 1966; Clusella Trullas et al., 2007), and usually represents the T_b at which several physiological processes function at an optimal level (Stevenson, 1985; Angilletta et al., 2002). As was pointed out by Basson and Clusella-Trullas (2015), if organisms start selecting a higher T_b in response to eventual warming, their RMR and EWL will increase. RMR is the minimal metabolism of an individual in a relatively quiescent state (Burton et al., 2011), and represents the rate at which organisms transform energy and materials (*sensu* Gillooly et al., 2001). An increase of RMR would involve higher energetic costs of maintenance, less available energy for reproduction and higher vulnerability to starvation (Dillon et al., 2010). In addition, higher T_b has consequences for an organism's water balance, increasing ventilation (with a concomitant higher respiratory water loss) and transpiration (as a result of lower skin resistance), the two principal components of EWL in squamates (Guillon et al., 2014; Dupoué et al., 2015).

Although ectotherms could cope with warming temperatures by changing their TPC (Ruiz-Aravena et al., 2014; Llewelyn et al., 2018) or their critical temperatures (Clusella-Trullas and Chown, 2014; Llewelyn et al., 2018) through acclimation plasticity, it is well known that acclimation responses could vary across populations and species (Barria and Bacigalupe, 2017; Barria et al., 2018; Llewelyn et al., 2018). For instance, animals could maintain or partially maintain their T_p, RMR and EWL across thermal changes, following the temperature compensation hypothesis (COMP; Clarke, 2003). Alternatively, in cold-adapted species, the acclimation response could result in higher RMR, T_p and EWL, when animals are acclimated to low temperatures compared with when they are acclimated to high temperatures, following the predictions of the colder is better hypothesis (CIB; Huey et al., 1999). Both hypotheses are particularly important in ectotherms living at high altitude, where the thermal amplitude is greater and most of the time, ambient temperature is lower than T_p measured in the laboratory. In seasonally variable environments, the CIB response could save energy whereas the COMP response will optimize performance, at the temperatures experienced during activity windows. Moreover, in a climate change scenario, through any of these acclimation responses, the reduction of EWL and RMR would help to maintain the water balance and would decrease the rate of food intake, partially counteracting the 'metabolic meltdown' (the decrease in the net energy gain) predicted for ectotherms in warmer environments (Huey and Kingsolver, 2019). However, whereas a COMP response could promote resilience by allowing individuals to maintain their activity time, metabolic rate and water balance, it could facilitate the exposure of the organism to extreme temperatures. In contrast, a CIB response would confer the capacity to conserve water and could avoid the risk of overheating, reducing the activity time window under warm conditions, which, simultaneously, could be detrimental for the net energy gain.

The leopard iguana *Diplolaemus leopardinus* (Werner 1898) (Leiosauridae), a rock-dwelling, sit-and-wait and oviparous lizard, inhabits the cold-arid Central Andes of Argentina. In these harsh environments, the leopard iguana behaves like a moderate thermoregulator, reaching T_b in the field near its T_p (Vicenzi et al., 2020). Individuals living in such seasonal and daily fluctuating environments require broader tolerance ranges and behavioral and physiological acclimation abilities to buffer that variation (Janzen, 1967; Bacigalupe et al., 2018; Kreiman et al., 2019). Here, we studied the extent to which individuals of *D. leopardinus* can adjust different traits to short-term periods of temperature change in an integrated way, to elucidate possible effects of warming on the species. The plasticity of the thermal preference is inseparable from the complex temperature dependence and acclimation responses of metabolism and water balance (Basson and Clusella-Trullas, 2015). Therefore, we evaluated the direction, magnitude and pattern of acclimation responses in thermal preference and TPCs of RMR and EWL. Because *D. leopardinus* inhabits cold and highly variable thermal environments, we expected lizards to respond following the CIB hypothesis. Therefore, we predicted that T_p, RMR and EWL would acclimate in the same direction and with similar magnitude, maximizing performance in the cold treatment.

MATERIALS AND METHODS
Study site
In December 2018 (late Austral spring), we captured 17 lizards (8 females, 6 males, 3 juveniles) by hand or lasso in the Angostura mountain valley (32.983°S, 69.333°W, WGS84, 2405 masl), in the Central Andes of Mendoza, Argentina. The Angostura valley is characterized by a cold, arid climate with a mean annual temperature of 6.1°C, a maximum temperature in summer of 29°C, and a minimum temperature in winter of −15.5°C. In this type of environment, lizards remain inactive from autumn until early spring. The mean annual precipitation of this site is 359 mm (Sileo et al., 2015), with two important peaks: one as rain in midsummer (February) and the other as snow in midwinter (July; Sileo et al., 2015). In this highly seasonal landscape, the leopard iguana shows a mean T_b of 26.69±1.18°C and a mean T_p of 30.85±0.26°C. Also, the mean operative temperature during the same period was 19.42 ±0.49°C (Vicenzi et al., 2020).

Animal maintenance
Once captured, lizards were transported to the laboratory located at the Instituto Argentino de Investigaciones de las Zonas Áridas (IADIZA, CCT Mendoza, CONICET), at 840 masl. The laboratory is located ca. 1500 m lower than the capture site, and has an increase of ca. 17% in oxygen availability versus that at the capture site. Individuals were maintained in a temperature (24±1°C) and photoperiod (12 h:12 h, dark:light) controlled room, with UV light (Sylvania® Reptistar T8 lamps, Osram Sylvania, Wilmington, MA, USA) on 8 h per day over a period of 7 days. Animals (N=17) were individually housed in terraria (32 cm width×45 cm length×27 cm height), provided with substratum and rocks collected at the capture site, with free access to a shelter and basking sites. Water was supplied *ad libitum*, and food (crickets, mealworms and *Blaptica* cockroaches) was provided every other day *ad libitum*. We recorded the sex for each lizard; because *Diplolaemus* species have inconspicuous sexual dichromatism, sex determination was based on examination of external morphology traits. For instance, males were identified by enlargement at the base of the tail caused by the hemipenes, and if the enlargement was not prominent (i.e. subadults), the hemipenes were manually everted to confirm sex. 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 for animal care.

Acclimation treatments
After 1 week in the laboratory, 9 individuals (3 adult males, 4 adult females, 2 juveniles) were acclimated to 20±2°C and 8 individuals (3 adult males, 4 adult females, 1 juvenile) were acclimated to 30±2°C experimental temperatures. The 20°C treatment reflected the mean operative temperature currently registered in the field during activity time of lizards (19.42±0.49°C; Vicenzi et al., 2020). Operative temperature represents the product of interactions between biophysical and morphological factors that influence the T_b of an ectothermic organism (Bakken and Gates, 1975; Hertz et al., 1993). The temperature selected for the warmer treatment is closer to the projected increases in frequency and duration of heatwaves predicted by the IPCC (2013) for the study area. We randomly assigned lizards to one of the two experimental temperatures for 14 days, and then we measured T_p, RMR and EWL. Afterwards, they were exposed to the alternative experimental temperature for 14 days, and again, the physiological traits were measured. Body mass did not statistically change as a result of the acclimation treatment ($F_{1,15.2}$=1.99, P=0.178; see below for details on analyses).

Physiological traits
Preferred temperature
To measure T_p (*sensu* Pough and Gans,1982; Hertz et al., 1993), we used a fiber-board terrarium (60 cm width×120 cm length×30 cm

height) partitioned into three lanes and partly covered by a lid on one end. The thermal gradient (23°C to 50°C) was achieved with a heliotherm (three 75 W halogen lamps suspended from the lid) and thigmotherm (four thermostat-controlled electric heat coils connected to four mica sheets) heat source (for more details, see Vicenzi et al., 2020). We affixed an ultrafine (44 ga) Type T thermocouple to the belly of each lizard using insulation material and surgical tape, to prevent the thermocouple from being dislodged during the experiment. The thermocouples were connected to an 8-channel data logger (Measurement Computing 1.2 kHz Data Acquisition Device, OMEGA® TC-08, ±0.5°C, Stamford, CT, USA). After 30 min, we recorded T_b at 1 s intervals for 140 min. We calculated the mean T_p and the mean temperature set-point (T_{set}, the interquartile range of T_p; *sensu* Hertz et al., 1993) from the population from the T_p, and the T_{set} obtained from each lizard (N=17). We also calculated the standard deviation (s.d.) of T_p to evaluate the precision in the selection of T_b.

RMR and total EWL rate

Three days after measuring T_p, RMR and total EWL were measured using standard flow-through respirometry and hygrometry methods. Metabolic rate was measured as the rate of carbon dioxide production ($\dot{V}_{CO_2}$) using a computerized open-flow respirometry system (Field Metabolic System, Sable Systems, Henderson, NV, USA). The incurrent air was passed through CO_2-absorbent granules of Baralyme® and H_2O-absorbent granules of Drierite® to remove carbon dioxide and water, and then entered a dark metabolic chamber (0.35 l). The chamber received air with a flow of 250 ml min^{-1} when we evaluated the adults, and 200 ml min^{-1} in the case of the juveniles (Naya et al., 2008), from a mass flow controller and through Bev-Aline tubing (Thermoplastic Processes Inc.). The excurrent air passed through the water vapor sensor and columns of Drierite®, before passing through CO_2 analyzers. The complete trial lasted 1 h. The output from the H_2O (kPa) and CO_2 analyzers (%) was digitized using a Universal Interface II and recorded on a personal computer using ExpeData® data acquisition software (Sable Systems). Our sampling interval was 1 s. We averaged the water vapor pressure and the CO_2 concentration of the excurrent airstream over a 10 min period after a steady state was reached (Tieleman et al., 2003). $\dot{V}_{CO_2}$ was calculated as $\dot{V}_{CO_2}=(F_{I_{CO_2}}-F_{E_{CO_2}})\times FR\times 60$, where $F_{I_{CO_2}}$ is the incoming fractional concentration of CO_2 from the metabolic chamber, $F_{E_{CO_2}}$ is the fractional concentration of CO_2 leaving it, and FR is the flow rate (ml min^{-1}) after STP correction (Lighton, 2008). EWL was calculated as EWL=FR×($P/R_w×T$), where FR is the flow rate after STP correction, P is the water vapor pressure of the air (Pa), T is the temperature of the dew-point hygrometer (K) and R_w is the gas constant for water vapor (461.5 J kg^{-1} K^{-1}; Lide, 2001).

We measured RMR and EWL at five T_b (20, 24, 28, 32 and 34°C) between 09.00 h and 19.00 h (daily activity time of leopard iguanas). We used a Peltier-effect cabin to achieve constant ambient temperatures during the measurements (PELT-5; range: −5 to 65°C, stability: 0.01 to 0.2°C, Sable Systems). The order of the trials was randomized. We measured body mass (BM) before the metabolic measurements using an electronic balance (±0.1 g), and we recorded cloacal T_b at the beginning and end of each trial using a TPK01 thermocouple attached to a TES 1312A digital thermometer (±0.01°C; TES® Electrical Electronic corp., Taipei, Taiwan). Before each measurement of RMR and TEWL, lizards were fasted for 48 h (Artacho et al., 2013).

Statistical analysis

Statistical analysis of data was carried out in R version 3.6.3 (http://www.R-project.org/). We fitted individual TPCs for RMR using the nlsLM function of the minpack.lm package (https://cran.r-project.org/web/packages/minpack.lm/index.html). From each curve under each acclimation treatment, we obtained the highest RMR (maximum $\dot{V}_{CO_2}$) and the T_b at which it occurred (T_{hRMR}). We used a linear mixed modeling approach to evaluate the effects of acclimation temperature, treatment order, sex, BM and T_b (this last one just for EWL) on T_p and its precision, highest RMR and T_{hRMR}, and EWL, while taking into account that we had repeated measurements for individuals (i.e. each individual was measured at both acclimation temperatures). The statistical significance of the random factors (i.e. individual ID) was assessed by likelihood ratio tests based on restricted maximum likelihood. For all traits, with the exception of EWL (EWL: χ^2_1=12.5, P<0.001), the random factor was not significant (in all traits χ^2_1=0, P=1). Therefore, for EWL, P-values for fixed effects were obtained using type III sums of squares based on the Satterthwaite approximation for denominator degrees of freedom (Kuznetsova et al., 2017). For the remaining traits, we employed a simple linear model with a backward model selection procedure to evaluate the change in residual sums of squares of a full model versus a nested model that does not include the predictor being evaluated. We started with a full model including all predictors and their interactions, and removed one predictor at a time, until we obtained a minimum adequate model. Statistical analyses were performed using *lme4* (Bates et al., 2015) and *lmerTest* (Kuznetsova et al., 2017) packages. Afterwards, we compared T_p from acclimated individuals with their T_p in field conditions obtained by Vicenzi et al. (2020) using a Pearson correlation test. Results are shown as means±s.e.m.

RESULTS

T_p was not related to treatment order ($F_{1,28}$=2.18, P=0.15), the interaction between acclimation and treatment order ($F_{1,26}$=0.15, P=0.7), or the interaction between body mass and acclimation ($F_{1,27}$=1.5, P=0.23). T_p was positively associated with body mass (slope±s.e. 0.08±0.02, $F_{1,30}$=16.51, P<0.001). It was 1°C lower in the warmer acclimation treatment ($F_{1,30}$=12.50, P<0.001) and 0.54°C lower in males ($F_{1,30}$=5.30, P<0.05; Fig. 1). The T_p of lizards acclimated at 20°C was positively correlated (ρ=0.60, $t_{1,15}$=2.94, P<0.01) with that registered in natural conditions (without acclimation), whereas T_p of lizards acclimated at 30°C was not (ρ=0.14, $t_{1,15}$=0.57, P=0.58).

The precision (s.d.) of T_p was affected by the warm treatment. Lizards that were first acclimated to 30°C showed higher precision of T_p than those acclimated first to 20°C, but lizards acclimated secondarily to 20°C exhibited more precise T_p that those acclimated first to 20 or 30°C ($F_{1,28}$=5.18, P<0.05). Males and females did not significantly differ in the precision of their T_p ($F_{1,27}$=0.73, P=0.40), although in smaller individuals (slope±s.e. 0.04±0.01, $F_{1,28}$=10.59, P<0.005) the s.d. of T_p was smaller (Table 1).

RMR showed a non-linear relationship with T_b, rising with T_b to a maximum level, and then dropping in a downwards curve. Data fitted a Briere equation (Briere et al., 1999). The temperature at which the highest RMR occurred (T_{hRMR}) was not affected by the interaction between body mass and acclimation treatment ($F_{1,26}$=0.01, P=0.91), the interaction between acclimation and treatment order ($F_{1,27}$=0.44, P=0.51), sex ($F_{1,28}$=0.90, P=0.35), order ($F_{1,29}$=1.02, P=0.32) or body mass ($F_{1,30}$=1.14, P=0.29). T_{hRMR} was almost 2°C lower in the 20°C acclimation treatment ($F_{1,31}$=4.77, P<0.05; Fig. 1) than in the 30°C treatment. RMR was
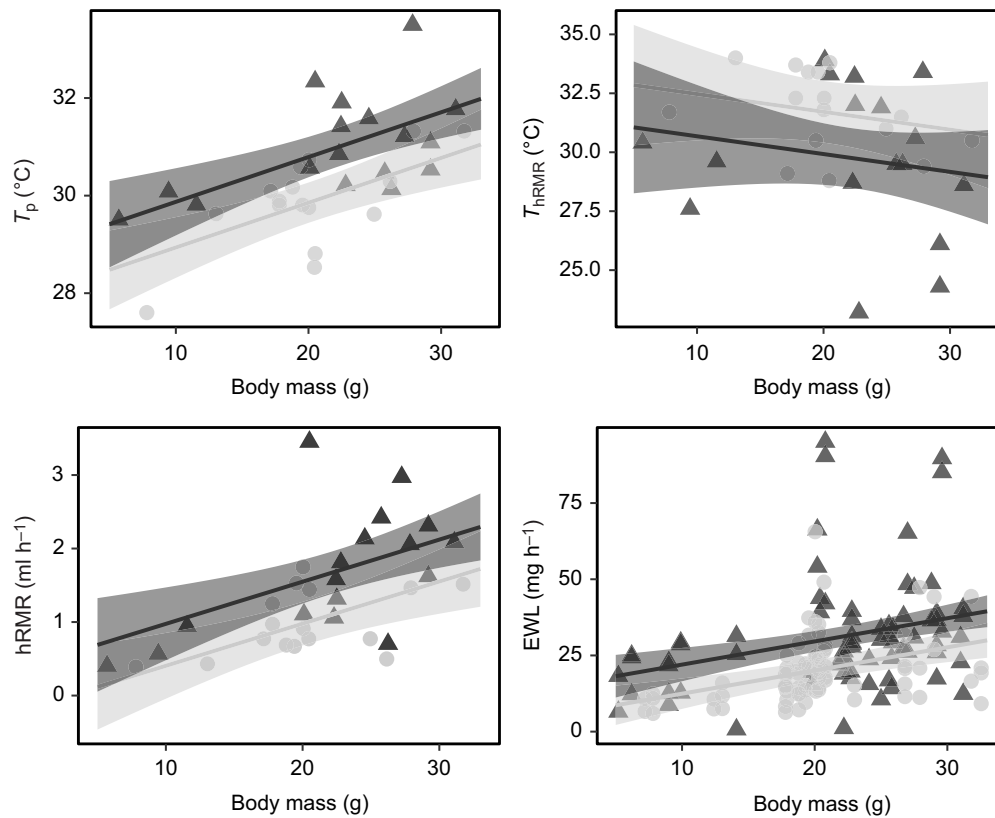
Fig. 1. Relationship between measured traits and body mass in *Diplolaemus leopardinus*. Preferred body temperature (T_p), body temperature of the highest resting metabolic rate (T_{hRMR}), highest magnitude of resting metabolic rate (hRMR, i.e. $\dot{V}_{CO_2}$) and evaporative water loss (EWL) showed a linear relationship with body mass for both temperature treatments (N=17 lizards). Dark gray and triangles represent the 20°C acclimation treatment and light gray and circles represent the 30°C acclimation treatment.

unaffected by the interaction between body mass and acclimation treatment ($F_{1,26}$=0.25, P=0.62) and sex ($F_{1,27}$=0.34, P=0.56), but increased with body size (slope±s.e. 0.03±0.32, $F_{1,28}$=6.11, P<0.05; Fig. 1). RMR ranged from 0.4 ml h^{-1} for the smallest lizards to 2.08 ml h^{-1} in the largest lizard in the cold treatment, and from 0.4 ml h^{-1} for the smallest lizards to 1.51 ml h^{-1} in the largest lizard in the warm treatment. RMR was also influenced by the interaction between acclimation and treatment order ($F_{1,28}$=6.25, P<0.05; Fig. 2). In fact, the order effect was only present when animals were acclimated first to the warm treatment and to second the cold treatment (Fig. 3). The group acclimated first to 20°C showed a mean hRMR of 1.11±0.15 ml h^{-1} in the colder treatment and 1.11±0.17 ml h^{-1} in the warmer one, whereas the lizards first acclimated to 30°C exhibited a mean hRMR of 0.84±0.11 ml h^{-1} in the warmer treatment and 2.49±0.20 ml h^{-1} in the colder one (Fig. 3).

Finally, EWL was not affected by the interaction between acclimation and treatment order ($F_{1,10.40}$=0.01, P=0.92), the interaction between body mass and acclimation treatment ($F_{1,134.20}$=1.06, P=0.30), order ($F_{1,146.63}$=1.27, P=0.26) or sex

($F_{1,12.10}$=4.40, P=0.06). The rate of EWL was positively related to body mass (slope±s.e. 0.01±0.004, $F_{1,10.29}$=5.74, P<0.05) and T_b (slope±s.e. 0.03±0.003, $F_{1,131.72}$=79.13, P<0.001), and was lower after the 30°C acclimation treatment ($F_{1,141.22}$=18.32, P<0.001; Fig. 2). At the highest temperature (34°C), EWL rate ranged from 0.4% of body mass per hour for the smallest lizards to 0.13% of body mass per hour for the largest lizards acclimated to the cold treatment, whereas it ranged from 0.16% of body mass per hour for the smallest lizards to 0.06% of body mass per hour for the largest lizards after exposure to the warm treatment.

T_p, RMR and EWL descriptive statistics are shown in Table 1. Allometric equations for T_p, T_{hRMR}, RMR and EWL are shown in Table S1.

DISCUSSION

Phenotypic plasticity is usually expected in heterogeneous thermal environments, because despite the energetic cost of production and maintenance of plastic traits, they allow individuals to deal with that variability (Ghalambor et al., 2007; Pigliucci, 2001). In the leopard iguana, a species inhabiting the highly seasonal environment of the

Table 1. Descriptive statistics for *Diplolaemus leopardinus* lizards exposed to 20 and 30°C acclimation treatments

Treatment	Sex	T_p (°C)	T_{set} (°C)	T_p s.d.	T_{hRMR} (°C)	hRMR (ml h^{-1})
20°C	Male	30.65±0.28	29.35±0.35 to 32.01±0.44	2.26±0.18	29.6±0.83	1.52±0.31
	Female	31.31±0.38	30.44±0.43 to 32.42±0.49	1.78±0.10	30.48±1.15	1.73±0.30
	Overall	31.00±0.25	29.93±0.31 to 32.27±0.32	2.00±0.11	29.75±0.73	1.69±0.21
30°C	Male	29.41±0.35	28.23±0.34 to 30.49±0.40	1.44±0.09	31.75±0.67	0.86±0.15
	Female	30.27±0.23	28.95±0.17 to 31.49±0.36	1.76±0.18	31.65±0.59	1.09±0.16
	Overall	29.86±0.22	28.61±0.20 to 31.01±0.29	1.61±0.11	31.07±0.43	0.99±0.01

Data are shown for preferred body temperature (T_p), set-point of preferred body temperature (T_{set}), preferred body temperature precision (T_p s.d.m.), body temperature of the highest value of resting metabolic rate (T_{hRMR}) and the highest magnitude of resting metabolic rate (hRMR, $\dot{V}_{CO_2}$). Means±s.e.m. of raw data are shown (N=17 lizards, 8 males and 9 females).
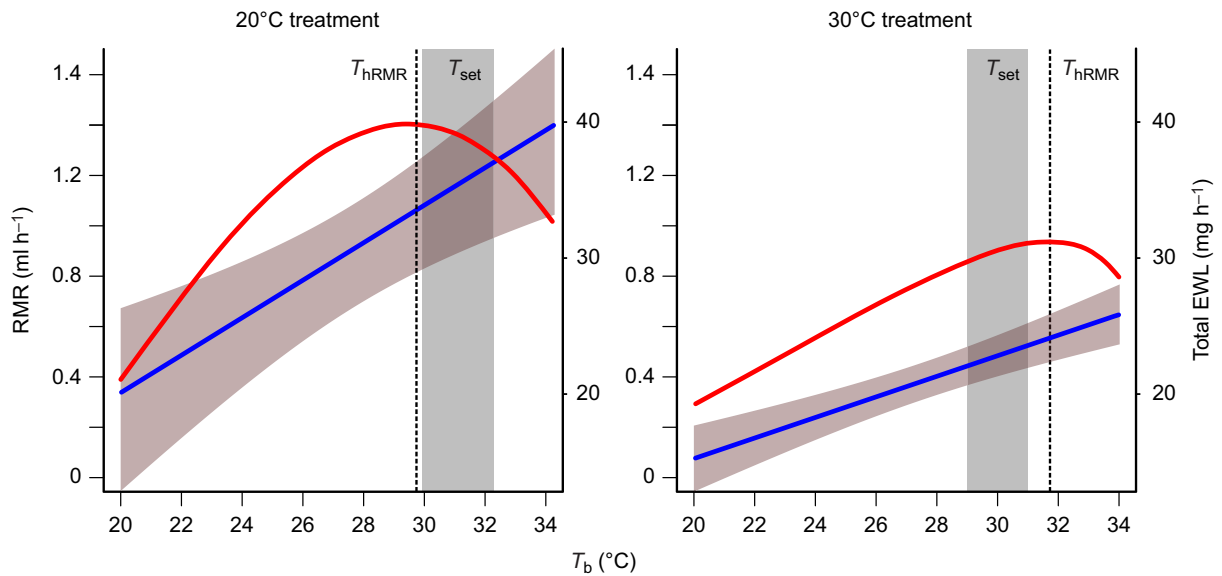
Fig. 2. Predicted population resting metabolic rate and EWL in relation to body temperature in *D. leopardinus*. Thermal performance curves (TPCs) of resting metabolic rate (RMR, i.e. $\dot{V}_{CO_2}$; red) and total EWL (blue) are shown for lizards (N=17) acclimated to 20 and 30°C. The dashed black line represents the body temperature at which hRMR was achieved, and the gray area represents the temperature set-point (T_{set}; 50% interquartile range of preferred body temperatures; 20°C treatment: 29.93–32.27°C; 30°C treatment 28.92–30.29°C). TPCs are shown for the global population, without discriminating the acclimation order effect (see Results). Statistical analysis was performed with the parameters obtained from the TPC of each individual.

high Andes, T_p, the T_b at which RMR is the highest, and EWL were adjusted and showed a behavioral–physiological interplay in the plastic response. In particular, our results show: (i) a reduction in T_p and EWL in the warmer treatment, (ii) an increment in T_{hRMR} under the same treatment (Table 1), (iii) the absence of a change in hRMR between treatments (Fig. 3), and (iv) a strong effect of the recent thermal history on the magnitude of the RMR.

Diplolaemus leopardinus is a cold-adapted species, as revealed by its relatively low T_p and low optimum temperature for bite force
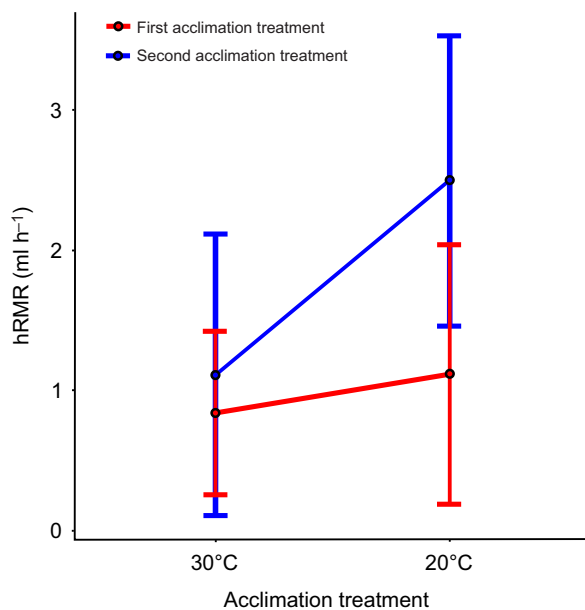

Fig. 3. Effect of acclimation order on hRMR of *D. leopardinus*. hRMR ($\dot{V}_{CO_2}$) obtained from each individual curve in relation to acclimation treatment (20 or 30°C) and acclimation order (first in red and second in blue) is shown (N=17). Data are means and 95% confidence intervals of hRMR of individuals.

in nature compared with other Andean lizards (Vicenzi et al., 2020). Furthermore, the higher values of T_p and EWL after acclimation to the 20°C treatment (i.e. the 'colder' treatment) show that activity and performance, such as locomotion and foraging activities, are favored in this lizard in colder environments (Rogowitz, 1996; Basson and Clusella-Trullas, 2015).

Lizards acclimated to the 30°C treatment showed lower T_p (29.86 ±0.22°C; Table 1, Fig. 1), following the predictions of the CIB hypothesis. Moreover, T_p in the 20°C treatment was correlated to T_p of individuals immediately after capture (without acclimation) as shown in Vicenzi et al. (2020). The plastic response of T_p could also be interpreted as an inverse acclimation or inverse compensation, because it reveals an opposite relationship between acclimation temperature and thermal preference (Tsuji, 1988a; Gvoždík et al., 2007). Inverse acclimation has been reported for *Sceloporus occidentalis* and *Phrynocephalus* lizards (Wilhoft and Anderson, 1960; Wang et al., 2013) and the salamander *Triturus dobrogicus* (Gvoždík et al., 2007). Inverse acclimation of T_p would be expected after exposure to warm temperatures because the margin between T_p and the critical thermal maximum in natural conditions is usually small (reviewed in Angilletta, 2009). Therefore, the selection of lower T_b represents a behavioral response to prevent further exposure of the organism to extreme temperatures (Feder and Pough, 1975; Gvoždík et al., 2007; Basson and Clusella-Trullas, 2015). However, in *D. leopardinus*, given that T_p, T_{hRMR} and EWL showed a similar response in the direction of their change, we hypothesize that the selection of a lower T_p is mostly related to a reduction in RMR and EWL under warm conditions.

The thermal RMR curve of leopard iguana in the laboratory revealed a short-term acclimation in T_{hRMR} and in the TPC pattern, following the COMP hypothesis. The T_b of the highest RMR partially compensated for the thermal change between treatments, being 1.95°C lower in the 20°C treatment (T_{hRMR}=29.75±0.76°C) than in the 30°C treatment (T_{hRMR}=31.7±0.41°C; Fig. 2), whereas the overall TPC pattern changed from a nearly symmetrical shape in

the 20°C treatment to a left-skewed one at 30°C (Fig. 2). Leopard iguanas acclimated to 30°C concentrated the maximum values of RMR in a narrow T_b range compared with the TPC of lizards acclimated to 20°C. This plastic response reduces the temperature sensitivity of RMR, which would minimize the increase in energy expenditure and water needs predicted for warming environments (Moffett et al., 2018).

The RMR acclimation response showed sensitivity to the individual's previous thermal experience. In particular, we only found RMR differences in those lizards acclimated to 20°C that had been first exposed to warm conditions (Fig. 3). Lizards initially acclimated to 30°C or to 20°C, and those acclimated second to 30°C (after the 20°C acclimation) reached similar values of RMR, whereas lizards acclimated second to 20°C (after the 30°C acclimation) showed a remarkably higher increment of 224% in the highest magnitude of RMR (Fig. 3). In agreement with the findings of Barceló et al. (2009), our results show that after acclimation to a warm thermal treatment, the response of RMR to cold exposure is exacerbated (Fig. 2). In line with this, thermal history and seasonality have important influences on the acclimation responses of RMR, as was recorded for *Cordylus oelofseni* (Basson and Clusella-Trullas, 2015), *Sceloporus* lizards (Tsuji, 1988a,b) and *Tupinambis merianae* (=*Salvator merianae*; Toledo et al., 2008), among others. In particular, Tsuji (1988a) reported that *Sceloporus* lizards from thermally heterogeneous environments, after 1 and 2 months of acclimation, differ in their RMR adjustments depending on the capture season (spring or autumn). Although our sample size was rather small, the reported patterns are clear and strengthen the notion that the acclimation ability of our study species is shaped by its recent thermal history. Moreover, contrary to our predictions, the highest magnitude of RMR remained constant when we compared short-term exposure to warmer or colder conditions (Fig. 3), but, when the recent thermal history involves a warm past, the thermal sensitivity of RMR increases in an extraordinary way, which would involve higher energetic costs of maintenance and higher vulnerability to starvation (Dillon et al., 2010).

EWL was positively affected by T_b, and showed a ca. 15% decrease in magnitude in the 30°C treatment compared with the 20°C treatment. This drop is partially associated with the change in the TPC pattern of RMR in response to acclimation. Lower RMR implies a decrease in oxygen demand, and consequently lower ventilation and a drop in respiratory water loss (Dmi'el, 1972). However, unlike the thermal metabolic curve, EWL continued rising along with T_b (Fig. 2). This increment in EWL occurred as a consequence of the use of cooling mechanisms, such as transpiration and the display of ventilatory behaviors (such as rapid breathing through the nares and breathing with an open-mouthed gape; Tattersall et al., 2006). Thermal plasticity of EWL in *D. leopardinus* is an important finding because it reveals the capacity to conserve water in warming conditions for this cold-adapted species. Despite the fact that leopard iguanas were provided with water *ad libitum* in both treatments, the EWL response to higher temperatures led to further behavioral and physiological water-conserving mechanisms: lower thermal preference, changes in the thermal sensitivity of RMR, as also reported in other reptiles (Dmi'el, 1972; Bradshaw, 1997; Davis and DeNardo, 2009; Guillon et al., 2014; Dupoué et al., 2015; Rozen-Rechels et al., 2019), and thermal acclimation. In terrestrial reptiles, water and thermal balance influence most biological processes, and their regulation shares behavioral and physiological responses influenced by functional trade-offs between them (Rozen-Rechels et al., 2019).

In species that inhabit cold and arid environments, water balance and thermoregulation are often in conflict, because the selection of higher T_b increases water loss by transpiration and ventilation (Köhler et al., 2011). Temperature and water constraints directly influence individual performance; for example, hydration state affects the optimal temperature and the shape of TPCs (Rozen-Rechels et al., 2019). Our results show how lizards are able through different mechanisms (i.e. changes in T_p, RMR, thermal acclimation) to cope with higher temperatures and, consequently, the dehydration risk.

The Central Andes of Argentina are among the regions considered to be particularly vulnerable to climate change impacts, as a result of the projected warming (between 3 and 4°C of the mean temperature) and the increase – in frequency and duration – of drought and heatwaves by the end of this century (IPCC, 2013; Barros et al., 2015). Model predictions indicate that this scenario will increase the time that lizards spend in shelters (Sinervo et al., 2010), thus reducing foraging opportunities (Sinervo et al., 2010; Sears et al., 2011). The increase in RMR and the lower foraging time, combined with the expected reduction in the abundance of arthropods (Deutsch et al., 2008; Hallmann et al., 2017; Lister and Garcia, 2018), will decrease the net energy gain of insectivorous lizards ('metabolic meltdown'; *sensu* Huey and Kingsolver, 2019). Moreover, environmental temperatures also influence water balance; and an organism's hydric condition influences its thermal biology, affecting individual performance (Rozen-Rechels et al., 2019). Our results suggests that in *D. leopardinus*, the recent thermal history plays a key role in shaping RMR. The highest magnitude and thermal sensitivity recorded for RMR did not show thermal acclimation in the short term, when lizards experienced cold or temperate environmental conditions in the recent past. This strengthens the concept of a future metabolic meltdown in ectotherms, because energy costs will increase at higher temperatures. Moreover, when lizards recently experienced steady warm conditions, we recorded an exacerbated increase in RMR during exposure to the 20°C treatment (which represents the mean operative temperature of our study site), revealing a strong effect of warming (Fig. 2). This could imply that after a short exposure to warm temperatures, as in a heat wave, subsequent RMR could be compromised as a result of a remarkable increment in its thermal sensitivity. We recorded plasticity of T_p, T_{hRMR} and EWL, which could partially counteract the hydric deficit predicted for arid lands (Field et al., 2012). Recent findings on our study species indicate an increase in bite force for individuals acclimated to 30°C (Vicenzi et al., 2020), which entails the ability to capture larger prey and the reduction of the time needed to manipulate them, improving its profitability (Herrel et al., 1996). Nevertheless, our present results are less optimistic: the contraction of the time window for activity arising from the selection of lower T_b, combined with the low thermal plasticity in the RMR under this temperature, will reduce the net energy gain and slow the growth and reproduction rates of the species (Huey and Kingsolver, 2019). Integrating behavioral and physiological traits provides a better understanding of the mechanisms involved in plastic responses, and their evolutionary trade-offs (Magozzi and Calosi, 2015), allowing stronger considerations about the vulnerability of *D. leopardinus* to climate change.

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Table S1. Allometric relationships of preferred body temperature (T_p), body temperature of the highest magnitude of resting metabolic rate (T_{hRMR}), highest magnitude of resting metabolic rate (hRMR), and evaporative water loss (EWL) with body mass in each acclimation treatment (20°C and 30°C).

Treatment	Trait	Intercept	Slope
20°C	T_p	29.3	0.08
	T_{hRMR}	30.96	-0.05
	hRMR	1.13	0.07
	EWL	13.44	0.80
30°C	T_p	27.48	0.12
	T_{hRMR}	33.96	-0.11
	hRMR	0.25	0.04
	EWL	6.24	0.69