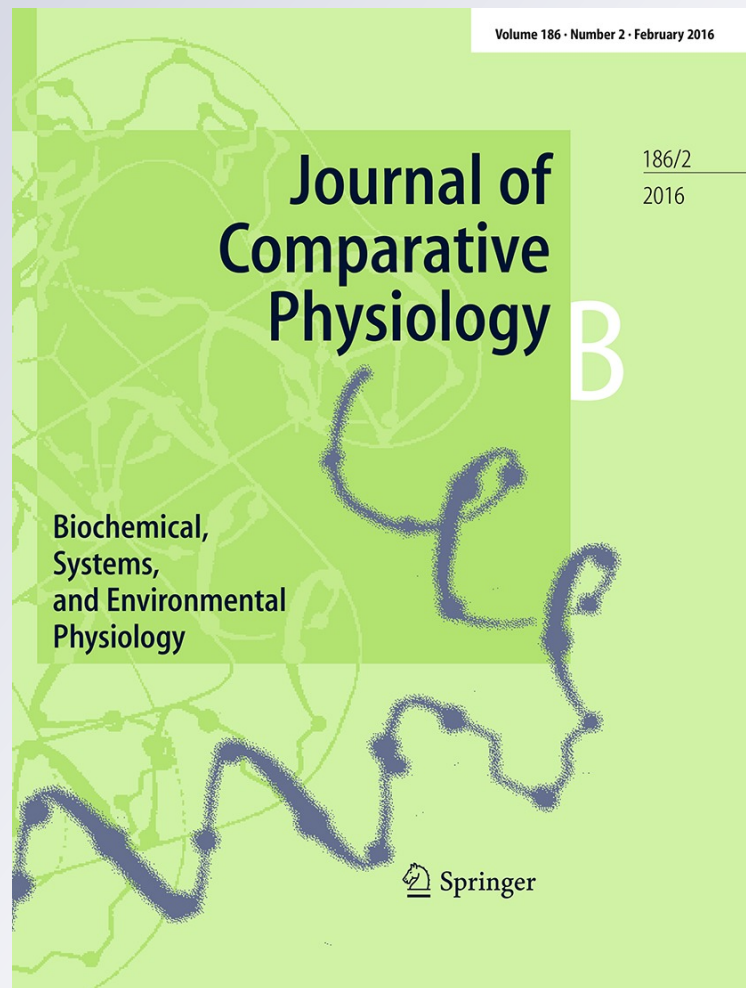*Vulnerability to climate warming of* Liolaemus pictus *(Squamata, Liolaemidae), a lizard from the cold temperate climate in Patagonia, Argentina*

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Vulnerability to climate warming of *Liolaemus pictus* (Squamata, Liolaemidae), a lizard from the cold temperate climate in Patagonia, Argentina

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Abstract The vulnerability of populations and species to global warming depends not only on the environmental temperatures, but also on the behavioral and physiological abilities to respond to these changes. In this sense, the knowledge of an organism's sensitivity to temperature variation is essential to predict potential responses to climate warming. In particular, it is interesting to know how close species are to their thermal limits in nature and whether physiological plasticity is a potential short-term response to warming climates. We exposed *Liolaemus pictus* lizards, from northern Patagonia, to either 21 or 31 °C for 30 days to compare the effects of these treatments on thermal sensitivity in 1 and 0.2 m runs, preferred body temperature (T_{pref}), panting threshold (T_{pant}), and critical minimum temperature (CT_{Min}). Furthermore, we measured the availability of thermal microenvironments (operative temperatures; T_e) to measure how close *L. pictus* is, in nature, to its optimal locomotor performance (T_o) and thermal limits. *L. pictus* showed limited physiological plasticity, since the acclimation temperature (21 and 31 °C) did not affect the locomotor performance nor did it affect T_{pref}, the T_{pant}, or the CT_{Min}. The mean T_e was close to T_o and was 17 °C lower than the CT_{Max}. The results suggest that *L. pictus*, in a climate change scenario, could be vulnerable to the predicted temperature increment, as this species currently lives in an environment with temperatures close to their highest locomotor temperature threshold, and because they showed limited acclimation capacity to adjust to new thermal conditions by physiological plasticity. Nevertheless, *L. pictus* can run at 80 % or faster of its maximum speed across a wide range of temperatures near T_o, an ability which would attenuate the impact of global warming.

Keywords Locomotor performance · Panting threshold · Critical temperature · Preferred temperature · Acclimation · Liolaemidae · Phenotypic plasticity · Climate change · Lizard · Patagonia

Introduction

A rise in environmental temperatures due to global warming can detrimentally affect an organism's physiological performance including growth, foraging, reproduction, immune capabilities, behaviors and competitiveness. Predictions of the effects of climate change species frequently rely on the analyses of the variation of locomotor performance with environmental temperatures (Angilletta 2009; Huey et al. 2012), since locomotion influences the fitness of individuals and is particularly subject to the pressures of natural selection (Snell et al. 1988; Bennett 1990; Jayne and Bennett 1990; Sinervo et al. 2000; Miles 2004; Cote and Clobert 2007; Zajitschek et al. 2012; Logan et al. 2013). Locomotion relates with dispersal or migration, feeding activities, predator avoidance, dominance behavior, and reproduction (Bennett 1980; Christian and Tracy 1981; Snell et al. 1988; Robson and Miles 2000; Perry et al. 2004). In this regard, the thermal sensitivity of lizards' locomotion becomes one of the most relevant locomotor parameters and can be calculated from performance curves

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defined by the optimal temperature (T_o) at which maximum speed is achieved (V_{max}), the thermal performance breadth (B_{80} = range of body temperature over which the lizard can run at 80 % or faster of its V_{max}) and the critical thermal minimum and maximum (CT_{Min} and CT_{Max}, respectively; Huey et al. 2012).

A short-term response to climate change by an organism or by a population may be physiological plasticity, and one of the main goals is to determine the potential for ectotherm species to exploit novel environments through shifts in the thermal performance curve (Huey and Kingsolver 1989, 1993). In this regard, thermal sensitivity of performance curves can vary depending on the duration of exposure to a particular temperature (Somero 2010). Such physiological plasticity is called acclimatization (due to natural changes) or acclimation (due to changes in a single factor in laboratory studies; Huey et al. 2012). Despite the importance of studies that investigate the effects of thermal acclimation, this kind of research is still scarce in lizards (Clusella-Trullas and Chown 2013).

The genus *Liolaemus* represent an appealing model to study the physiological plasticity and the potentiality to adapt to climate change, because it has shown high evolutionary radiations into novel environments and versatility in thermal biology (Medina et al. 2009, 2012; Moreno Azócar et al. 2012; Pincheira-Donoso et al. 2013); reproduction (Ibargüengoytía 2008) and performance (Bonino et al. 2011, 2015). In particular, the species *L. pictus*, widespread from moderate to high altitudes (529–1600 m asl) in the Andean-Patagonian forests of Chile and Argentina, has been shown to be plastic in its reproductive biology (showing biennial to triennial cycles; Ibargüengoytía and Cussac 1996) and growth dynamics (Gutiérrez et al. 2013). However, a recent study focusing on the distributions of populations of *L. pictus*, the disposability of their operative temperatures, and the expected rise of temperature in the coming years, forecasts that 15 % of the populations could become extinct by 2080 due to the increment in the hours of restriction (Kubisch et al. 2015). *Liolaemus pictus* is a heliothermic lizard which shows low intraspecific variation in field and preferred body temperatures (Ibargüengoytía and Cussac 2002; Gutiérrez et al. 2010; Kubisch et al. 2015) and behaves as a moderate to poor thermoregulator (Gutiérrez et al. 2010). The temperature at which *L. pictus* reaches its maximum speed when they run long distances (T_o) is similar to their activity field body temperature (T_b; Kubisch et al. 2011).

Our goal is to test whether *L. pictus*, in nature, is close to optimal performance temperature and maximum thermal limits, and whether *L. pictus* responds to increasing temperatures by acclimation, especially to determine if it is able to adjust its thermal sensitivity for running performance and thermal physiology (preferred body temperature, panting threshold and minimum critical temperature) when exposed to higher environmental temperatures.

Materials and methods

Field work and specimens

Field work was carried out on the shore Lake Nahuel Huapi (41°07′S and 71°20′W, 771-m asl) in San Carlos de Bariloche (Río Negro Province, Argentina). The site is located adjacent to The Andes, in the Patagonian Phytogeographic Province, and is characterized by cold temperate climate with monthly mean temperatures from 2 to 15.5 °C, precipitation from 22 to 140 mm and intense prevailing winds from the West (National Weather Service).

Liolaemus pictus lizards were caught by noose during January 2010, from 10 to 19 hs. Immediately after capture, the body temperature (T_b) was measured using a thermocouple inserted 1 cm inside the cloaca (Catheter probe TES TP-K01, 1.62 mm diameter) and connected to a TES 1302 thermometer (TES Electrical Electronic Corp., Taipei, Taiwan, ±0.01 °C). Once in the laboratory, body mass (with Ohaus, Scot Pro ±0.01 g) and snout-vent length (SVL; vernier calliper ±0.02 mm) were recorded. Lizards were classified as juveniles or adults considering the minimum adult size for *L. pictus* as 49 mm SVL (Ibargüengoytía and Cussac 1996) and the sex was determined by the presence of pre-cloacal pores in males.

Acclimation treatments

Thirty-five animals were randomly assigned to each of the two acclimation temperatures in constant temperature chambers set at 21.34 °C (±0.04) and 31.26 °C (±0.05). The 21 °C acclimation trial was chosen as an intermediate temperature between the mean maximum environmental temperature (19.54 ± 0.68), recorded 30 days before capture, and the mean air temperature recorded when lizards were active in the field (24.6 °C: Ibargüengoytía and Cussac 2002; 23.2 °C: Gutiérrez et al. 2010), and considering the lowest temperature that a lizard can experience during the experiment and remain active to feed. The 31 °C acclimation trial resembles the mean T_b recorded in the field in this study (31.96 °C ± 0.41), similar to the findings of Gutiérrez et al. (2010) for the same population (32.6 °C), and of Ibargüengoytía and Cussac (2002) for a nearby population (32.2 °C) and also correspond to the highest temperature to maintain lizards in the experiments without subjecting them to thermal stress.

In each acclimation chamber the temperature was recorded every 2 min for 8 days to monitor the mean and variability of acclimation temperature (datalogger HOBO

Table 1 Gender and sexual maturity, mean (±SE) and range of body mass (g) of males, females, and juveniles of both 21 and 31 °C acclimated group, n = sample size

Acclimation trials (n)	Gender and sexual maturity (n)	Mean (±SE) and range of body mass (g)	Mean (±SE) and range of body mass (g) overall
21 °C (17)	Females (6)	6.30 (±0.52) 4.34–7.71	5.81 (±0.48) 2.51–8.80
	Males (9)	6.20 (±0.66) 3.54–8.80	
	Juveniles (2)	2.60 (±0.09) 2.51–2.69	
31 °C (15)	Females (9)	4.87 (±0.24) 3.97–6.33	4.95 (±0.41) 2.40–8.36
	Males (6)	6.12 (±0.65) 3.73–8.36	
	Juveniles (2)	2.52 (±0.12) 2.40–2.63	

Table 2 Ranges and means (and their standard error, ±SE) of the body temperature (T_b) for long and sprint runs at the four temperature trials: 16 °C (low temperature trial), 22 °C (medium temperature trial), 30 °C (high temperature trial), and 37 °C (very high temperature trial)

Temperature trials (°C)		Acclimation trials (°C)	Mean (±SE) and range of T_b (°C)
Long runs	16	21	16.46 (±0.13) 15.60–17.10
		31	16.57 (±0.10) 15.30–17.20
	22	21	22.61 (±0.14) 21.70–23.50
		31	23.29 (±0.13) 22.50–24.30
	30	21	31.04 (±0.21) 29.60–32.50
		31	30.59 (±0.17) 29.20–31.90
	37	21	37.65 (±0.15) 36.60–38.90
		31	37.69 (±0.29) 34.70–9.30
Sprint runs	16	21	16.10 (±0.26) 13.50–17.20
		31	16.16 (±0.17) 14.80–17.20
	22	21	23.71 (±0.17) 22.50–25.00
		31	23.09 (±0.35) 19.90–26.00
	30	21	30.12 (±0.31) 28.10–32.60
		31	29.82 (±0.27) 28.10–31.40
	37	21	37.41 (±0.13) 36.40–38.40
		31	37.88 (±0.48) 35.90–40.80

Pendant). Also, the temperatures of acclimation were compared with the environmental temperatures recorded every hour from the nearest weather station located at Aeropuerto Bariloche (41°6′S and 71°7′W) for 30 days before capture.

Sex and sexual maturity of lizards of the different treatments are described in Table 1. Mean body mass between the acclimation groups was compared. During the acclimation period, each lizard was placed individually in a terrarium (15 × 20 × 20 cm) with a sand floor and a shelter and was provided with water and mealworm larvae (*Tenebrio molitor*) ad libitum. Lizards were maintained with a 12L:12D photoperiod using 40-W cool fluorescent tubes for a 30-day period of acclimation prior to any experimental test. Body mass was measured before and after the

acclimation period and no individual lost more than 15 % of body mass during captivity.

After 30 days of acclimation, the following trails were carried out in successive days: locomotor performance in long (LR) and sprint runs (SR) at four body temperature ranges, preferred body temperatures (T_{pref}), critical thermal minimum (CT_{Min}), and panting threshold. At the end of each trial, animals were returned to their respective acclimation temperatures. Water, but not food, was supplied in-between trials.

Locomotor performance trials

Running trials were carried out on a racetrack 1.50 m long and 0.08 m wide, with a compacted floor of fine sand and a shelter at the end. Each lizard performed one sprint run trial and one long run trial per day with a minimum rest period of 4 h between trials. The long runs trial (LR), which described sustained speed, consisted of three consecutive 1 m runs, and the sprint run trial (SR), which described acceleration, included five consecutive 0.2 m runs. The runs were filmed using a Sony DCR-SR 45 video camera, recorded in NTSC with an error of ±0.03 frame per second following the methodology of Fernández et al. (2011), Fernández and Ibargüengoytía (2012), and Kubisch et al. (2011). All videos were processed using the program AVS Video ReMaker (±0.033 s) to determine the running speed. Before each run, the lizard's body temperature (T_b) was measured using a thermocouple inserted 1 cm inside the cloaca (Catheter probe TES TP-K01, 1.62 mm diameter) and connected to a TES 1302 thermometer (TES Electrical Electronic Corp., Taipei, Taiwan, ±0.01 °C).

Both types of runs were conducted at four T_b ranges in four consecutive days hereafter referred to as 16 °C (low temperature trial), 22 °C (medium temperature trial), 30 °C (high temperature trial), and 37 °C (very high temperature trial; Table 2). To avoid confounding the effects of temperature and test sequence on performance, individuals were randomly assigned to the different temperature trials. Lizards were placed in an environment with the specified

temperature 1 h prior to the run, following the methods of Angilletta et al. (2002a) and Kubisch et al. (2011). For each temperature trial, only the speed of the fastest run performed by each lizard was included in the analysis.

Estimation of preferred body temperatures

Lizards were placed individually in an open-top terrarium ($200 \times 20 \times 27$ cm^3) with a sand substratum and a thermal gradient (20–50 °C) produced by a line of four infrared lamps overhead (one 250 W, two 150 W and one 100 W) each adjusted in height to create a linear temperature gradient. The body temperature of each lizard was measured every 10 min for 5 h using an ultra-thin (0.08 mm) catheter thermocouple located approximately 1 cm inside the cloaca and fastened to the base of the lizard's tail to keep the thermocouple in position during the experiment. For each individual, we estimated the mean (T_{pref}), the range, and the set-point temperature range as the central 50 % of all body temperatures selected in the laboratory. We compared the mean T_{pref} of the acclimated lizard with the data of the unacclimated *L. pictus* published in Gutiérrez et al. (2010).

Critical thermal minimum (CT$_{Min}$)

To determine the critical thermal minimum (CT$_{Min}$), lizards were placed individually in a plastic transparent box ($15 \times 10 \times 5$ cm^3) at −10 °C. Body temperature was measured every 15 s using an ultra-thin (0.08 mm) catheter thermocouple located approximately 1 cm inside the cloaca and fastened to the base of the lizard's tail. Lizards were observed throughout the experiment and the body temperature at which the individual was no longer able to right itself when placed on its back was recorded and considered as CT$_{Min}$.

Panting threshold and critical thermal maximum (CT$_{Max}$)

In order to determine the panting threshold, each lizard was placed in an open-top terrarium ($15 \times 20 \times 20$ cm) with a sand floor and an infrared 150-W lamp 40 cm overhead. The body temperature was monitored every 15 s as described for the thermal gradient observations. Each lizard was carefully observed throughout the experiment. The panting threshold was considered as the body temperature at which the individual first presented vigorous movements of escape or opened its mouth to lose heat by evaporation (sensu Kubisch et al. 2011). Lizards were then removed from the heat source and allowed to recover.

A subsample of eight individuals captured on December 2012 was used only to estimate critical thermal maximum (CT$_{Max}$). The same experiment used for panting threshold was performed, except that each individual was made to stay longer until it was unable to right itself when it was placed on its back. The individuals were then immediately cooled down to allow recovery.

Thermal sensitivity

To quantify the speed thermal sensitivity, we used the software Table Curve 2D v5.01 to create performance curves for each individual. Critical minimum (CT$_{Min}$) and critical maximum temperatures (CT$_{Max}$) were used as the extreme values of the curves (speed equal to 0 m/s). The model selection for performance curves was done using the Akaike index (AICc) which measures the goodness of fit of the model to the data penalized by the number of estimated parameters (Burnham and Anderson 2002). The speed data of each lizard were fitted to a quadratic model in correspondence with one of the models proposed by Angilletta (2006). We estimated the maximum speed (V_{max}), the optimum temperature (T_o), and the performance breadth (B_{80}) of LRs and SRs at both acclimation temperatures (21 and 31 °C) using the performance curves obtained from each individual. Additionally, for comparative purposes, the performance curves of the experiments performed on the same lizards before acclimation (unacclimated) were calculated using the data published in Kubisch et al. (2011).

Comparisons before and after the acclimation period

We estimated the performance thermal parameters (V_{max}, T_o, and B_{80}) of the same lizards before acclimation and we compared them with the parameters estimated after 30 days in acclimation chambers using the paired *t* test when data followed parametric assumptions or the Wilcoxon Signed Rank Test as a non-parametric option. Moreover, we compared the T_{pref}, CT$_{Min}$, and T_{pant} means between acclimated and unacclimated lizards of different individuals from the same site of capture (data published in Gutiérrez et al. 2010; Kubisch et al. 2011) using the *t* test when data followed parametric assumptions or the Mann–Whitney Test as a non-parametric option.

During acclimation and/or experiments, lizards were maintained with asepsis and without any contact with other animals. After all the experiments, lizards were released at their precise capture location, which had been geo-referenced using a GPS device (Garmin Map 60C Sx).

Operative temperatures and indices to estimate vulnerability to global warming

Physical lizard models were made to determine the operative temperatures (T_e) in the micro-environments that *L. pictus* can use (potential micro-environments) (Hertz

1992). The T_e was obtained to determine the spatial and temporal heterogeneity of thermal microenvironments for thermoregulation. We also used the values for T_e to assess the potentiality of lizards to attain body temperatures within the range of optimal physiological temperatures (Bakken et al. 1985; Bakken 1992; Dzialowski 2005; Williams et al. 2008). We built hollow PVC models (1.5 × 8 cm section) painted dull gray (18 %) to mimic body size, reflectance and shape of *L. pictus* T_bs. Each model was connected to a thermocouple and sealed at the ends with silicone (Fastix®). The models were validated using a live lizard (Pearson product moment correlation: $r = 0.914$; $n = 11{,}211$; lizard T_bs = $-5.339 + 1.193 ×$ Physical model; Kubisch et al. 2015). In order to capture the T_e variation present on the study site, eight thermal models connected to data loggers (HOBOTEMP® H8, 4-External Channel) were deployed in micro-environments potentially used by *L. pictus*. Three physical models were placed in the sun, two models were placed in the shade (such as under a bush), and three models were placed in shelters (such as under rocks). Values for T_e were recorded with a sampling interval of 2 min between 900 and 1900. We sampled T_e during the reproductive season for *L. pictus*, which extends from November to January (Ibargüengoytía and Cussac 1996).

We determined the thermal safety margin (TSM) for physiological performance as the difference between the T_o and mean T_e, and the warming tolerance (WT) as the difference between mean T_e and CT_{max} sensu Deutsch et al. (2008) and Logan et al. (2013), in order to know how close is this *L. pictus* population to their thermal optimal performance and thermal limits in nature and its potential to access to an above-ground thermal refuge.

Statistical analysis

We used the statistical software programs Sigma Stat 3.5®, Sigma Plot 10.0®, and Table Curve 2D v5.01 for statistical analysis. The differences between the mean values of two samples were analyzed using paired t tests when the same individuals were compared and unpaired t tests when different individuals were compared. The assumptions of normality for parametric procedures were checked using the Kolmogorov–Smirnov Test. When the assumptions of normality were not met, we used equivalent nonparametric tests such as Mann–Whitney Test for the comparison of two independent samples, Wilcoxon Signed Rank Test for the comparison of two dependent samples, or Kruskal–Wallis One-Way Analysis of Variance on Ranks for the comparison of more than two independent samples. The means are presented with the standard error ($\pm$SE) and when the assumptions of normality failed we presented the median. The significance level used for all statistical tests was $P < 0.05$.
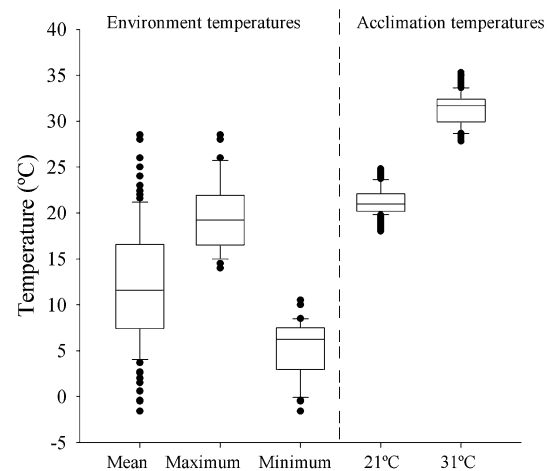

Fig. 1 Box plots indicate median, 25th and 75th percentile, and the extremes of the maximum, minimum, and mean of air temperatures in the natural environment during 30 days before capture (obtained from the nearest weather station, Bariloche Airport), and the air temperature at which lizards were exposed in the acclimation trials (21 or 31 °C)

Results

Comparisons between environmental temperatures in nature and during acclimation

Temperatures from both 21 and 31 °C acclimation trials were significantly non-normal (Kolmogorov–Smirnov, 21 °C acclimation temperatures: K-S Dist. = 0.111 and 31 °C acclimation temperatures: K-S Dist. = 0.099, $P < 0.001$; weather station temperatures: K-S Dist. = 0.056, $P > 0.200$). For this reason we performed a non-parametric test to compare acclimation temperatures and the mean air temperatures recorded in nature during 30 days before capture. The median air temperature of the 31 °C acclimation trial was 31.69 °C and was higher than the 21 °C acclimation trial performed at a median of 20.99 °C. Both acclimation temperatures were significantly higher than the median air temperature recorded at the nearest weather station (11.10 °C) (Kruskal–Wallis: $H_2 = 20{,}004.23$, $P < 0.001$; Dunn's Method: 31 °C acclimated vs nature: $Q = 23.31$; 30 °C acclimated vs 21 °C acclimated: $Q = 42.23$; 21 °C acclimated vs nature: $Q = 7.57$, $P < 0.05$; Fig. 1).

Effect of acclimation temperature

There were no differences in body mass between the acclimation groups (t test, $t_{33} = -0.018$, $P = 0.985$). Speeds at 16, 22, 30, and 37 °C temperature trials were not different between lizards exposed to 21 °C and those exposed to 31 °C in LR or SR (Table 3; Fig. 2).

Table 3 Comparisons of lizards speed between the group exposed to low temperature (21 °C) and the group exposed to high temperature (31 °C) in long (LR) and sprint runs (SR); in each temperature trial: 16 °C (low temperature trial), 22 °C (medium temperature trial), 30 °C (high temperature trial), and 37 °C (very high temperature trial)

Temperature trial (°C)	Run type	Acclimation trials (n)	Mean ($\pm$SE) or median speed	t test or Mann–Whitney
16	LR	21 °C (11)	0.30 ($\pm$0.01)	$t_{21} = -0.78$ ($P = 0.45$)
		31 °C (12)	0.31 ($\pm$0.02)	
	SR	21 °C (13)	0.33 ($\pm$0.02)	$t_{19} = -2.12$ ($P = 0.05$)
		31 °C (8)	0.42 ($\pm$0.04)	
22	LR	21 °C (12)	0.48	$U = 138.50$ ($P = 0.52$)
		31 °C (12)	0.50	
	SR	21 °C (17)	0.45	$U = 148.50$ ($P = 0.90$)
		31 °C (17)	0.45	
30	LR	21 °C (12)	0.45	$U = 130.00$ ($P = 0.26$)
		31 °C (12)	0.54	
	SR	21 °C (16)	0.44	$U = 160.50$ ($P = 0.39$)
		31 °C (17)	0.64	
37	LR	21 °C (12)	0.48	$U = 133.00$ ($P = 0.34$)
		31 °C (12)	0.56	
	SR	21 °C (14)	0.60 ($\pm$0.06)	$t_{28} = -0.59$ ($P = 0.56$)
		31 °C (16)	0.65 ($\pm$0.07)	

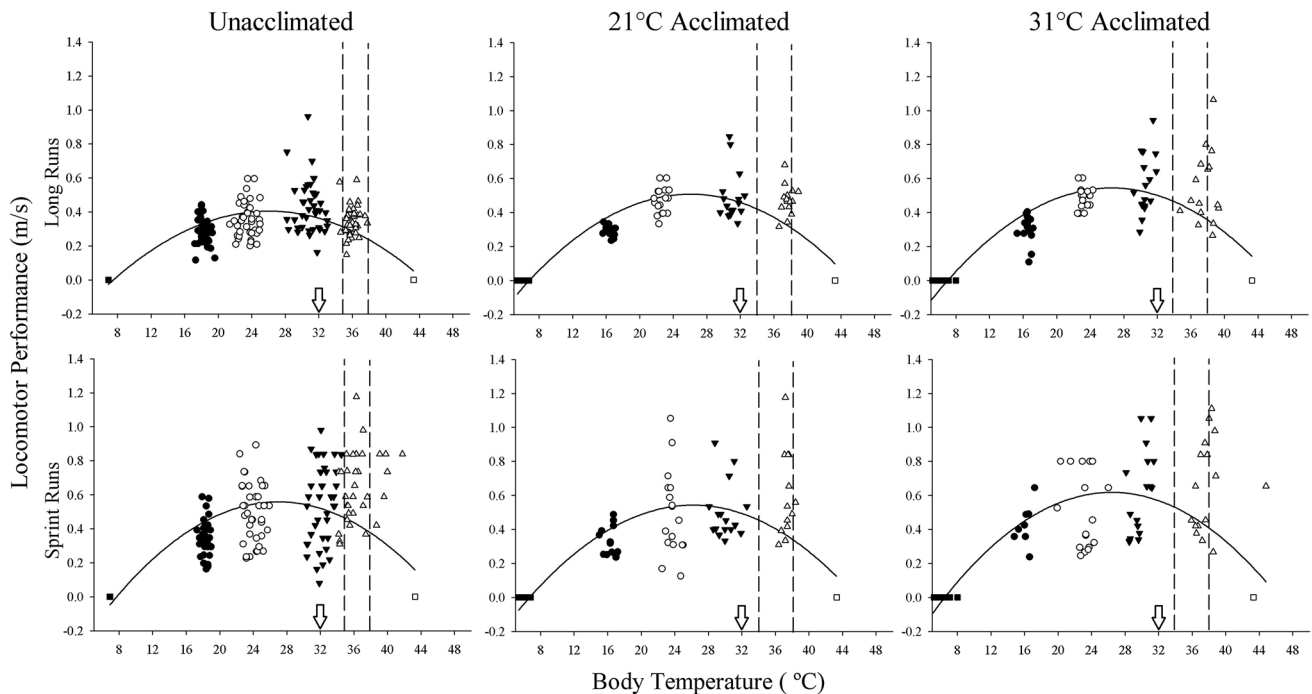Means (and their standard error, $\pm$SE) or median speed and sample size (n) are also indicated

Fig. 2 Relationship between speed and body temperature on long and sprint runs of *Liolaemus pictus* before and after a 30 day acclimation at 21 and 31 °C. Each temperature trials are differentiated: 16 °C (*black circles*), 22 °C (*white circles*), 30 °C (*black triangles*), and 37 °C (*white triangles*), minimum critical temperature (*black square*), and maximum critical temperature (*white square*). *Vertical dashed lines* indicate the set-point range of preferred body temperature. The mean body temperature on the field (31.9 °C) is indicated with an *arrow*

Table 4 Mean (and their standard error, ±SE), range, and set-point range of preferred body temperature in laboratory (T_{pref}), panting threshold, and thermal critical minimum of *Liolaemus pictus* of unacclimated lizards of the same population (data published in Gutiérrez et al. 2010 and Kubisch et al. 2011) and after the acclimation period (at 21 and 31 °C)

	T_{pref}			Panting threshold		Thermal critical minimum	
	Mean (°C; ±SE)	Range (°C)	Set-point range (°C; ±SE)	Mean (°C)	Range (°C)	Mean (°C)	Range (°C)
Unacclimated ($n = 27$) (Gutiérrez et al. 2010)	36.60 (±0.39)	32.6–39.3	34.60 (±0.61)–37.95 (±0.29)	–	–	–	–
Unacclimated ($n = 15$) (Kubisch et al. 2011)	–	–	–	42.81 (±0.11)	40.2–44.4	6.91 (±0.42)	4.0–10.0
Acclimated 21 °C ($n = 8$)	36.07 (±0.99)	32.2–39.6	34.34 (±1.32)–38.47 (±0.33)	42.96 (±0.25)	41.1–42.3	6.04 (±0.11)	5.4–6.8
Acclimated 31 °C ($n = 14$)	36.09 (±0.51)	31.2–38.7	34.26 (±0.72)–38.44 (±0.38)	43.04 (±0.79)	42.3–45.0	6.07 (±0.25)	4.8–8.0

Table 5 Estimated mean maximum running speed, mean optimal temperature (T_o), mean performance breath (B_{80}), and range performance breath for lizards before and after acclimation at 21 and at 31 °C for long and sprint runs

Groups	Run trial (n)	T_o (°C; ±SE)	Maximum speed (m × s^{-1}; ±SE)	Performance breath B_{80} (±SE)
Before acclimation	LR (40)	25.97 (±0.08)	0.40 (±0.01)	16.45 ± 0.040 (17.74 ± 0.08–34.19 ± 0.09)
	SR (39)	26.78 (±0.18)	0.56 (±0.02)	16.99 ± 0.14 (18.28 ± 0.12–35.27 ± 0.25)
21 °C acclimated group	LR (16)	26.01 (±0.14)	0.51 (±0.02)	17.17 ± 0.08 (17.43 ± 0.12–34.60 ± 0.16)
	SR (16)	26.22 (±0.37)	0.56 (±0.03)	17.50 ± 0.20 (17.47 ± 0.29–34.97 ± 0.47)
31 °C acclimated group	LR (17)	26.45 (±0.25)	0.55 (±0.03)	17.69 ± 0.28 (17.60 ± 0.29–35.29 ± 0.29)
	SR (17)	26.36 (±0.27)	0.62 (±0.05)	17.63 ± 0.28 (17.54 ± 0.20–35.17 ± 0.39)

Sample size (n) and standard errors (±SE) are also indicated

The T_{pref}, T_{pant}, and CT_{Min} were not different between lizards exposed to 21 °C and lizards exposed to 31 °C (t test; T_{pref} $t_{20} = 0.02$, $P = 0.985$; T_{pant} $t_{20} = 0.21$; $P = 0.837$; CT_{Min}: $t_{24} = -0.11$; $P = 0.912$; Table 4). T_o did not differ between lizards exposed to 21 °C and lizards exposed to 31 °C in long and sprint runs (LR: Mann–Whitney, $U = -184.000$, $P = 0.087$, $n = 31$; SR: t test, $t_{31} = -0.29$, $P = 0.772$). Similarly the V_{max} was not different between lizards exposed to 21 °C and lizards exposed to 31 °C in long or sprint runs (LR: t test, $t_{31} = -1.16$, $P = 0.254$; SR: Mann–Whitney, $U = -156.000$; $P = 0.482$, $n = 31$).

Paired comparison of lizards before and after the acclimation trials

There were not differences in T_o when comparing the same individuals before and after 30 days in captivity (LR 21 °C acclimated: paired t test, $t_{14} = -0.62$, $P = 0.544$; LR 31 °C acclimated: paired t test, $t_{15} = -1.39$, $P = 0.185$; SR 21 °C acclimated: Wilcoxon Signed Rank Test, $W = 70.000$, $P = 0.05$, $n = 15$; SR 31 °C acclimated: paired t test, $t_{15} = 0.67$, $P = 0.510$; Table 5).

For long runs, both acclimation treatments increased V_{max} after acclimation (LR 21 °C acclimated: paired t test, $t_{14} = -4.87$; LR 31 °C acclimated, $t_{15} = -5.26$;

$P < 0.001$). In sprint runs there were not differences in V_{max} before and after 30 days of captivity (SR 21 °C acclimated: paired t test, $t_{14} = -1.24$, $P = 0.234$; SR 31 °C acclimated: paired t test, $t_{15} = -0.47$, $P = 0.647$; Table 5).

For long runs, in both acclimation treatments B_{80} increased after acclimation (LR 21 °C acclimated: paired t test, $t_{14} = -5.86$; LR 31 °C acclimated: Wilcoxon Signed Rank Test, $W = 134.000$, $P < 0.001$, $n = 32$). In sprint runs there were not differences in B_{80} before and after 30 days in captivity (SR 21 °C acclimated: paired t test, $t_{14} = -0.874$, $P = 0.397$; SR 31 °C acclimated: paired t test, $t_{15} = -1.45$, $P = 0.168$; Table 5).

Field body temperature, operative temperatures, and indices to estimate vulnerability to global warming

The mean field body temperature was $31.96 ± 0.41$ °C and varied from 25.5 to 39.9 °C. The mean operative temperature was 25.58 °C and varied from 8 to 45 °C (Fig. 3). The mean critical thermal maximum (CT_{Max}) was $43.29 ± 0.50$ °C. Therefore, the warming tolerance for *L. pictus* was 17.71 °C, and the thermal safety margin for physiological performance in LR was 0.43 °C and in SR 0.64 °C.
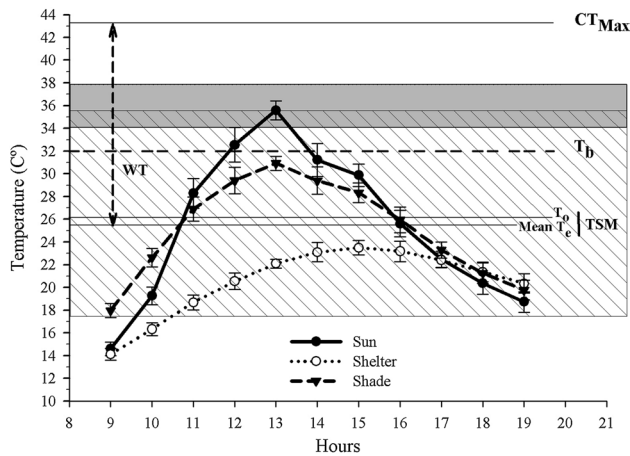
Fig. 3 Operative temperature (mean ± standard error) every hour during activity span, obtained from plastic models placed on different thermal microenvironments. The mean operative temperature (T_e), the critical thermal maximum (CT_{Max}), the mean body temperature in field (T_b), and the optimal performance temperature (T_o) of *Liolaemus pictus* are indicated with *horizontal lines*. The warming tolerance (WT) and the thermal safety margin for physiological performance (TSM) are indicated with *arrows*. The *gray rectangle* represents the set-point range of the preferred body temperature in laboratory that corresponds to the 25 and 75 % quartiles and the *rectangle* with *diagonal stripes* corresponds with the thermal performance breadth (B_{80} = range of body temperature over which the lizard can run at 80 % or faster of its maximum speed)

Discussion

Species exposed to variable seasonal environments can show different performance curves as a result of physiological plasticity (Stillman 2003; Schulte et al. 2011). However *L. pictus*, which lives in a cold temperate region with high daily and annual amplitude of environmental temperature, showed limited plasticity to acclimation in locomotor performance in long and sprint runs. A similar response has been observed in another Patagonian lizard, the gecko *Homonota darwinii*, whose maximum locomotion speed was not affected by a 62-h period of acclimation to different temperatures (18–19, 22–23, 27–28, and 32–33 °C; Aguilar and Cruz 2010). Overall, *L. pictus* data revealed limited plasticity in its thermal biology, showing similar T_o, T_{pref}, T_{pant}, and CT_{Min} between the group exposed to constant low temperatures (21 °C) and the group exposed to constant high temperatures (31 °C).

This is in agreement with the conservative character of some thermal biology traits such as T_{pref} in the genus *Liolaemus* (Labra 1998; Medina et al. 2009, 2012; Moreno Azócar et al. 2012), and the T_o in the lineomaculatus group (Bonino et al. 2011). In particular, *L. pictus* has shown similar T_{pref} values among populations localized at different altitudes (Gutiérrez et al. 2010) or different latitudes (Kubisch et al. 2015). In contrast, the thigmothermic

desert lizard from California, *Xantusia vigilis*, shows plasticity; individuals exposed to 30 °C had higher preferred, minimum critical, and maximum critical temperatures than individuals exposed to 20 °C (Kaufmann and Bennett 1989).When we compared the locomotor performance curves before and after acclimation, we observed that *L. pictus* improved their maximum speed and the B_{80} range was broader in long runs after acclimation. The great variability of air temperatures that this species experiences in nature (Fig. 1) has probably favored the existence of a wide range of isoenzymes associated with diverse physiological processes. In consequence, in laboratory with constant and higher temperatures, *L. pictus* can adjust its locomotor performance to new conditions. It is known that some proteins appear to play an important role in response to fluctuating temperatures, whereas others respond more strongly to constant temperatures (Podrabsky and Somero 2004). In contrast, *Liolaemus sarmientoi*, adapted to a harsh cold temperate environment, decreases locomotor performance in long and sprint runs after acclimation to a higher and constant temperature (21 °C) than the temperature they normally experience in their environment (average temperature during the active period October–March: 12.1 °C; Fernández and Ibargüengoytía 2012). Although, the acclimation temperatures for both *L. pictus* and *L. sarmientoi* species were included in the range of environmental temperatures, the acclimation treatments exposed them to a longer duration of exposure to one of the highest temperatures they may experience in their natural environment. The thermal variance seems to impact in thermal tolerances and performance in other ectotherm groups. For example, the thermal variance affects organismal survival, and population growth in *Drosophila melanogaster* (Bozinovic et al. 2011a) and locomotor performance in *Bungarus multicintus* (Ji et al. 2007).

Liolaemus pictus showed the lowest optimal temperatures for locomotor performance among the liolaemids studied (from 30 to 36 °C, Bonino et al. 2011; Fernández et al. 2011). Often, the T_o values match the preferred body temperatures (T_{pref}) obtained in the laboratory (Martin and Huey 2008). However, in lizards of cold temperate environments like Patagonia, the optimal temperatures for performance are often lower than the preferred body temperature (Ibargüengoytía et al. 2007; Fernández et al. 2011; Bonino et al. 2011). The estimated optimal temperatures in *L. pictus* were lower than the set-point range of T_{pref} (33.29–35.90 °C; Gutiérrez et al. 2010), suggesting that running performance is optimized to live in cooler environments. Whereas the T_{pref} probably results from selection to maximize other physiological variables such as endurance, digestion (Waldschmidt et al. 1986; Angilletta et al. 2002b), reproductive cycle (Beal et al. 2014) or hearing capacity (Werner 1976). Alternatively, current temperature

preference may be a primitive character inherited from a more benign environmental condition in the past (Medina et al. 2012).

Warming could affect lizards by reducing the frequency of optimal operative temperature for activity, by limiting the availability of microhabitats for the population or the percentage of territory that lizards could effectively use to perform vital activities such as feeding, dispersal, and breeding (Tewksbury et al. 2008; Sinervo et al. 2010; Logan et al. 2013). When the temperature increases and performance declines, lizards tend to seek refuges such as shady burrows and stop their activities thereby avoiding overheating but at the cost of lost hours of activity (Grant and Dunham 1988; Sinervo et al. 2010). However, activity rates are not always coupled to locomotor performance (Gunderson and Leal 2015). This is the case for *Anolis cristatellus* in which activity rate seems to be more sensitive to temperature than is locomotion, and lizards depress the activity rates at temperatures in which sprint performance remains high (Gunderson and Leal 2015). In *L. pictus* when analyzing hours of restriction of activity following the model of Sinervo et al. (2010) we found that 15 % of the populations of *L. pictus* were predicted to be extinct by 2080 (Kubisch et al. 2015).

In ectotherms, there is a tendency to increase thermal tolerance ($CT_{MAX} - T_e$) with latitude (Deutsch et al. 2008; Bozinovic et al. 2011b; Araújo et al. 2013; Weeks and Espinoza 2013; Bozinovic et al. 2014; Bozinovic and Pörtner 2015). In this sense, the warming tolerance was lower in four tropical *Anolis* species (WT = 3.1 to 7.8 °C; Logan et al. 2013) than in *L. pictus* from the cold temperate environment of Patagonia (WT = 17.71 °C). However, the other important index of vulnerability to climate warming, the thermal safety margin ($T_o - T_e$), suggests that the environmental temperatures are already close to the physiological optimal temperature in *L. pictus* with thermal safety margin (TSM) of 0.43–0.64 °C. The low TSM in *L. pictus* underscores the point that with only a small increment in temperature the running performance decreases more than in the four tropical *Anolis* species, with TSM ranging from 1.5 to 5.5 °C (Logan et al. 2013). Nevertheless, *L. pictus* can run at 80 % or more of its V_{max} around a wide range of temperatures near T_o ($B_{80} = 17$ °C), which would attenuate the impact of global warming on performance. Furthermore, the B_{80} resembles the heterogeneity of thermal microenvironments, rather than the mean, and would provide *L. pictus* a "buffer range of temperatures" to achieve a high locomotor performance (Fig. 3). In this sense, it is important to consider the variability of T_e more than the average (Bozinovic et al. 2011a; Rezende et al. 2014; Camacho et al. 2015).

One would expect that tropical lizards would be more affected by climate change than lizards from cold temperate environments in Patagonia. Strikingly, our results point out that a temperature increase could significantly affect, in the long term, the whole-organism physiological traits in *L. pictus*, since the optimum temperatures for locomotion (26–27 °C) are currently very close to the mean T_e (25.6 °C) and lower than the mean body temperature (31.9 °C) they achieve through thermoregulation in their natural environment. In addition, because *L. pictus* showed limited acclimation capacity in locomotor performance and other thermal physiological parameters (T_{pref}, T_{pant}, and CT_{Min}), the results highlight constraints in their ability to respond to a new thermal condition by plasticity. However, the heterogeneity of available thermal microenvironments in the undergrowth of the forest they inhabit together with the possibility to respond with a wider performance breadth could attenuate the impact of warmer climate.

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