



RESEARCH PAPER

Thermal physiology responds to interannual temperature shifts in a montane horned lizard, *Phrynosoma orbiculare*

Saúl F. Domínguez-Guerrero^{1,2,3} | Brooke L. Bodensteiner¹ | Alexis Pardo-Ramírez⁴ | David R. Aguillón-Gutiérrez⁵ | Fausto R. Méndez-de la Cruz² | Martha M. Muñoz¹

¹Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut

²Laboratorio de Herpetología, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, México

³Posgrado en Ciencias Biológicas, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, México

⁴Facultad de Ciencias Biológicas, Universidad Juárez del Estado de Durango, Gómez Palacio, Durango, México

⁵Centro de Investigación y Jardín Etnobiológico, Universidad Autónoma de Coahuila, Viesca, Coahuila, México

Correspondence

Saúl F. Domínguez-Guerrero and Brooke L. Bodensteiner, Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06511.

Email: sauldguerrero@gmail.com (S. F. D.-G.) and brooke.bodensteiner@yale.edu (B. L. B.)

Abstract

As climate change marches on, rapidly rising temperatures shatter records every year, presenting ever-growing physiological challenges to organisms worldwide. Ectotherms rely on behavioral and physiological plasticity to contend with environmental fluctuations. Nonetheless, our understanding of thermal plasticity has been largely limited to laboratory settings. Here, we test whether aspects of thermal physiology respond to interannual shifts in thermal environment in a natural population of *Phrynosoma orbiculare*, a montane horned lizard, from Hidalgo, Mexico. At our field site, 2019 was markedly warmer than the year that preceded it. We detected population-level increases in three key thermal physiological traits: preferred temperature, the critical thermal minimum, and the critical thermal maximum. Thus, thermal phenotypes appear to shift in tandem in response to environmental fluctuations. A subset of individuals were resampled across years, allowing insight into plastic shifts within an organism's lifetime. We detected parallel increases in these lizards for the preferred temperature and the critical thermal minimum, but not for the critical thermal maximum. Our results support a growing body of literature indicating that preferred conditions and cold tolerance can be highly labile over the course of an organism's lifetime, whereas hardening over shorter time periods is more common for heat tolerance. Given that heat tolerance increased at the population-level, but not in resampled individuals, it is possible that rapid evolution occurred due to temperature increases. In short, physiological shifts can be observed in natural populations over relatively short timespans, and these shifts might reflect a combination of evolutionary and acclimatory responses.

KEYWORDS

body temperature, critical thermal limits, phenotypic plasticity, physiological acclimatization, preferred body temperature, thermal physiology

1 | INTRODUCTION

Temperature is one of the most ubiquitous variables affecting biological processes (Angilletta, 2009; Gillooly, Brown, West, Savage, &

Charnov, 2001; Somero, Lockwood, & Tomanek, 2017). Temperature increase due to human activities presents a global threat to biodiversity, but the magnitude of its effects is unequally distributed across the tree of life (Sala et al., 2000; Thomas et al., 2004).

Saúl F. Domínguez-Guerrero and Brooke L. Bodensteiner contributed equally to this study.

Rising temperatures present unique challenges to ectotherms such as lizards, which rely on external environment to regulate their body temperature (Huey et al., 2012; Paaajmans et al., 2013). These challenges are further magnified in montane and tropical species, as these types of organisms are especially sensitive to temperature changes and, in many cases, are already functioning near their upper physiological limits (Deutsch et al., 2008; Huey et al., 2009). Concurrently, the pace and magnitude of climate change is predicted to be particularly severe in tropical regions (Williams, Jackson, & Kutzbach, 2007). Some estimates, for example, predict massive global declines in lizard populations in this century due to rising temperatures, particularly in the tropics (Huey et al., 2009; Huey, Losos, & Moritz, 2010; Sinervo et al., 2010); when such organisms are restricted to progressively warming mountaintops, they may find themselves on an “escalator to extinction” (e.g., Freeman, Scholer, Ruiz-Gutierrez, & Fitzpatrick, 2018).

However, organisms are not exclusively at the whim and mercy of their thermal environments. Through adjustments in their behavior and/or physiological limits, organisms can compensate (at least partially) for thermal fluctuations in their environments (Huey, Hertz, & Sinervo, 2003; Huey et al. 2012; Kearney, Shine, Porter, & Wake, 2009; Moritz & Agudo, 2013; Seebacher, White, & Franklin, 2015). Many ectotherms are highly precise thermoregulators, and are able to efficiently seek out their preferred thermal conditions even under fluctuating environmental conditions (Huey, 1982; Muñoz & Losos, 2018; Seebacher, 2005; Seebacher & Franklin, 2005). Physiological acclimatization (i.e., plastic adjustments in physiological traits) can also help organisms better match their physiology to their local thermal conditions (Gunderson & Stillman, 2015; Pintor, Schwarzkopf, & Krockenberger, 2016; Seebacher, 2005). Within an organism's lifetime, for example, upper and lower critical thermal limits (CT_{max} and CT_{min} , respectively) can be higher during the summer than the winter for many lizard species (Ballinger, Hawker, & Sexton, 1969; Larson, 1961; Smith & Ballinger, 1994; Spellerberg, 1972a, 1972b). Thus, studies addressing how thermal biology responds to environmental fluctuations are of key conservation interest, but studies focused on variation in natural populations remains relatively scant (Chevin, Lande, & Mace, 2010; Merilä & Hendry, 2014; Muñoz & Moritz, 2016). The previous 5 years set global records for temperatures (NOAA, National Centers for Environmental

Information, 2020). This unprecedented march toward a warmer world provides an opportunity to examine whether and how organisms use behavioral and physiological adjustments to dynamically respond to changing conditions.

The goal of our study was to investigate whether thermal physiology responded to environmental temperature increases between 2018 and 2019 in a natural population of the horned lizard, *Phrynosoma orbiculare*. This Mexican lizard is restricted to montane habitats, suggesting it may be particularly vulnerable to extinction (Dubey & Shine, 2010, 2011). At our study site in Hidalgo, Mexico (elevation = 2,500 m) temperatures were 1°C warmer in 2019 than in 2018 (Table 1), providing the opportunity for behavioral and physiological adjustments in *P. orbiculare*. In late summer 2018, we measured four key thermal traits: (body temperature [T_b], the preferred body temperature [T_{pref}], the critical thermal maximum [CT_{max}], and the critical thermal minimum [CT_{min}]). These traits describe how ectotherms like lizards interact with their thermal environments (Angilletta, 2009; Huey, 1982). We then remeasured those traits 1 year later in August–September 2019. Given higher environmental temperatures in 2019, we predicted increases in all four traits. Using a mark-recapture approach, we were able to resample a subset of individuals across years. This allowed us to estimate within-individual shifts in thermal physiology to indirectly infer whether population-level physiological shifts might reflect evolution (i.e., heritable intergenerational changes), phenotypic plasticity (i.e., intragenerational changes), or some combination of both. We predicted that any population-level differences in thermal traits should also be evident in recaptured individuals. Any phenotypic shifts at the population-level not matched by shifts in recaptured individuals suggest (but do not prove) that an evolutionary change might have occurred.

2 | METHODS

2.1 | Study organism, field sites, and body temperature measurements

The mountain horned lizard, *P. orbiculare*, is a viviparous lizard species distributed across high elevations (plateaus and mountains between ~1,400 and ~3,400 m above sea level) in the Sierra Madre

TABLE 1 Mann–Whitney *U* test of differences in climatic data (minimum, mean, and maximum daily air temperatures [°C] and mean daily precipitation [mm]) between years

Climate data	Year	Mean ± SE	Mann–Whitney <i>U</i> value; <i>p</i> value
Mean daily temperature	2018	12.7 ± 0.17	<i>U</i> = 58,267; <i>p</i> = .003
	2019	13.53 ± 0.16	
Mean maximum daily temperature	2018	20.07 ± 0.21	<i>U</i> = 54,478; <i>p</i> < .001
	2019	21.39 ± 0.21	
Mean minimum daily temperature	2018	5.43 ± 0.18	<i>U</i> = 64,869; <i>p</i> = .540
	2019	5.70 ± 0.16	
Mean daily precipitation	2018	1.69 ± 0.28	<i>U</i> = 66,515; <i>p</i> = .970
	2019	1.68 ± 0.26	

Note: Data were gathered from a weather station ~4 km away from the field site in Hidalgo, Mexico. *p*-values < .05 are presented in bold font.

Oriental and Occidental in the northern and central Mexico (Sherbrooke, 2003). Our focal population is located in an induced grassland on the periphery of a pine-oak forest (20.064°N, -98.201°W; 2,500 m) in the locality of El Encinal, municipality of Cuauhtepc de Hinojosa, Hidalgo, México. We performed field work (with two or three people at a time) in an area of 0.06 km² during 3 weeks between August 6 and September 12, 2018 and during 2 weeks from August 25 to September 8, 2019. During these periods we measured body temperatures from 08:00 to 18:00 hr (activity time of lizards in the study site; Pardo-Ramírez, 2019). We manually captured individuals and immediately (<10 s after the capture) measured their cloacal temperature (body temperature; T_b) using a type K thermocouple ($\pm 0.1^\circ\text{C}$ precision) connected to a digital handheld thermometer (Fluke 51-II®). Using the same thermocouple, we then measured the temperature of the substrate where lizards were observed perching (substrate temperature; T_s) and the air temperature ~5 cm above the substrate (air temperature; T_a).

2.2 | Measurement of critical thermal limits and preferred temperatures

On the same day of capture, lizards were transported to a field laboratory (~200 m from the study site with natural light and a maintained temperature of ~20°C). Following our previous work (Domínguez-Guerrero et al., 2019), we built a thermal gradient arena that consisted of a wooden box (100 cm long, 100 cm wide, and 30 cm tall), filled with 2 cm of sediment from the study site and divided into 10 tracks. At one extreme of thermal gradient, we suspended one 75 W bulb at a height of 25 cm above the gradient's ground. This resulted in a thermal gradient ranging from ~20°C on the lower end to ~50°C at the hotter end. After being given a night to rest, we placed lizards individually into arena lanes from 08:00 to 17:00 hr. At each hour mark during the experiment, we measured body temperature of lizards with the same thermocouple and digital thermometer used in field work. Lizards were hand-captured and body temperature measured as quickly as possible (<10 s). T_{pref} was estimated as the average of the central 50% of temperatures measured during this experiment (Hertz, Huey, & Stevenson, 1993).

After the T_{pref} experiment, we placed each lizard into a plastic container (25 cm diameter and 30 cm height) to estimate their critical thermal maximum, CT_{max} . This metric describes the upper limit of ectotherm locomotion (Spellerberg, 1972a). Following established methods (Muñoz et al., 2014), we placed a 90 W bulb 40 cm above the container, and steadily increased lizard's body temperature by 1°C/min (warming and cooling rates were estimated post hoc by measuring total temperature change/total experiment length). When lizards initiated evaporative cooling through panting, we began flipping them onto their back every 20 s and encouraged them to right themselves by prodding their thigh using a pair of blunt tweezers. When the lizard failed to right itself within 15 s, we recorded their body temperature as CT_{max} . Following the CT_{max} experiment, we immediately removed lizards from the heat source, cooled them

down to room temperature, and offered them water ad libitum. On the next day we placed each lizard into a rectangular container (23 cm long, 16 cm wide, and 8 cm tall) to estimate their critical thermal minimum, CT_{min} . This metric describes the lower limit of ectotherm locomotion (Spellerberg, 1972a). We placed the container on a bed of ice to decrease body temperature by a rate of 1°C/min (Muñoz et al., 2014). We flipped lizards onto their backs every 20 s. We recorded CT_{min} as the temperature at which lizards failed to right themselves after 15 s of encouragement. When we finished CT_{min} experiments, we allowed lizards to return to their preferred temperature in the thermal gradient for 1 hr. Finally, we weighed (± 0.01 g) and measured each lizard (snout-to-vent length [SVL] ± 1 mm), registered their sex, and recorded their age category. We considered adult females to have SVLs > 66 mm and adult males to have SVLs > 65 mm (Hernández-Navarrete, 2018). Animals with SVLs smaller than these cut-offs were categorized as juveniles. In anticipation of our follow-up field season in 2019, in 2018 we permanently marked lizards with three beads on the tail (Figure 1) following the methods of Fisher and Muth (1989). Weather station data, including precipitation mean, maximum, and minimum daily temperatures were obtained from the Servicio Meteorológico Nacional, México. These data are derived from the station AHUPB, located in Ahuazotepec, Puebla, Mexico (20.041°N, -98.174°W), which is approximately 4 km away from the field site. We used weather station data to characterize general weather patterns between 2018 and 2019 (Servicio Meteorológico Nacional, 2020).

2.3 | Statistical analyses

For the subset of lizards that we sampled in both 2018 and 2019, we compared thermal traits using a paired t test performing separate tests for each trait. We also used a paired t test to test for differences between microenvironmental measures (T_a and T_s) between years for these resampled individuals. To test for differences in the



FIGURE 1 Male *Phrynosoma orbiculare* with individual bead tag on tail for identification at the field site during the data collection period [Color figure can be viewed at wileyonlinelibrary.com]

macroenvironmental measures (mean, maximum, and minimum daily air temperature and precipitation) we used a Mann-Whitney rank sum test because the data were non-normally distributed. To compare population means of physiological traits between years we performed a mixed linear model with lizard ID as a random effect to account for repeat measures between years. Physiological traits (T_b , T_{pref} , CT_{min} , and CT_{max}) were designated as the dependent variables, and we included log-transformed SVL and T_a as continuous predictors and year, sex, and age class were categorical predictors. The microenvironmental measures (T_a and T_s) were highly correlated ($r = .72$; $p < .0001$), therefore, we only include T_a in the model as a predictor.

3 | RESULTS

3.1 | Variation in the thermal environment between years

At our field site the mean daily air temperature and the mean maximum daily air temperature were hotter in 2019 by approximately 0.83°C and 1.32°C respectively. However, we did not observe any differences in precipitation or the mean minimum daily temperature between years (Table 1). The lowest temperatures recorded at the weather station were -5.5°C in 2018 and -5.0°C in

2019. Meanwhile the hottest temperature recorded in 2018 and 2019 were 28.5°C and 33.5°C, respectively. Thus, not only did the mean maximum daily temperature increase, but there was also a 5°C increase between the absolute hottest temperature recorded between years. Air and substrate temperatures taken at the time and location of capture were hotter in 2019 (Figure 2). Specifically, there was approximately a 4.7°C increase in mean substrate temperature between years (mean \pm standard deviation [SD]: 2018 = 24.98 \pm 5.68; 2019 = 29.65 \pm 6.43; $p < .001$), and a 3.3°C increase in the air temperature (mean \pm SD: 2018 = 21.65 \pm 4.31; 2019 = 24.96 \pm 3.67; $p < .001$) at the time of capture between years.

3.2 | Lizard behavior and physiology

We collected a total of 48 individuals of *P. orbiculare* across both years, of which 20 were females and 28 were males (Table S1). Population means for all traits (body temperature, preferred temperature, heat tolerance, and cold tolerance) were higher in 2019 than in 2018 (Table 2 and Figure 3). At the population-level the largest magnitude increase between years was in mean field measured body temperature and preferred body temperature; specifically, body temperatures were, on average, 2.4°C warmer and the preferred body temperature was 2.3°C higher. Meanwhile, lower critical limit increased by 1.2°C and the upper thermal limit increased

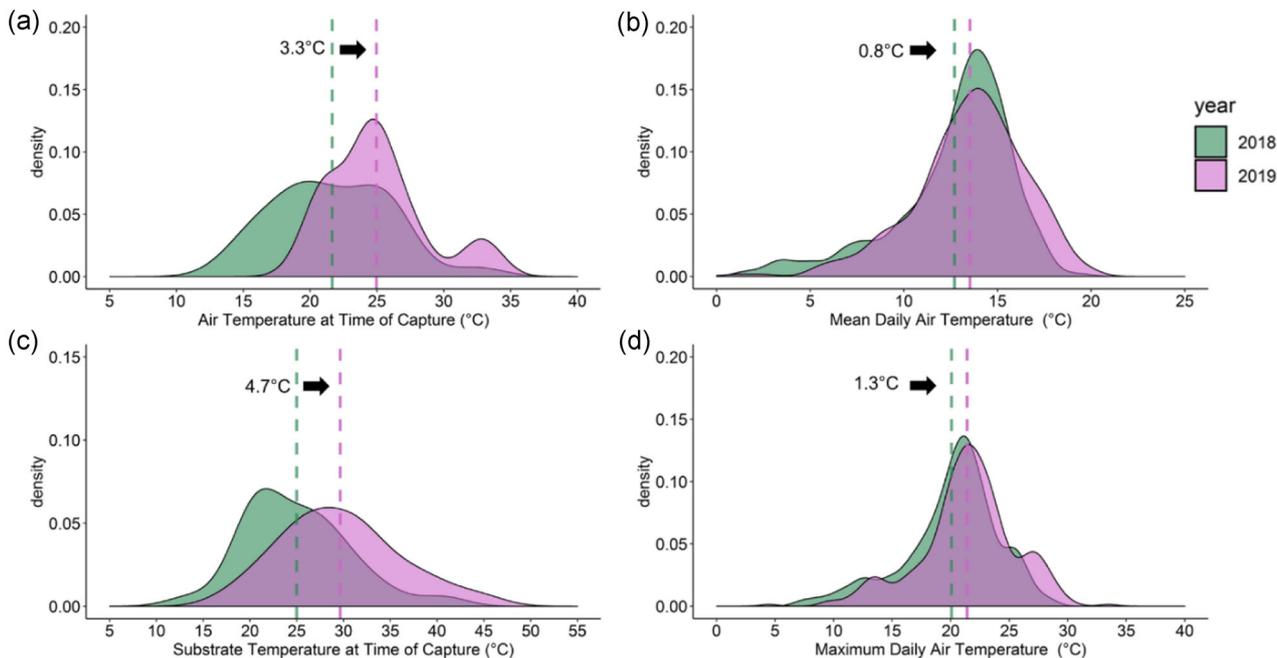


FIGURE 2 Distributions of macroclimatic (mean and maximum daily air temperatures gathered from nearby weather stations) and microclimatic conditions (air and substrate temperature at site of lizard capture) at the field site in Hidalgo, Mexico. (a) Air temperature at time of capture. (b) Mean daily air temperature. (c) Substrate temperature measured at the time of lizard capture and (d) Maximum daily air temperature. The thermal distributions for each variable are color coded by year, with measurements from 2018 given in green and measurements from 2019 shown in purple. The dashed lines (also color coded by year) denote the mean of each temperature distribution. The arrows denote the direction of temperature change from 2018 to 2019, and the magnitude of the corresponding change is also given [Color figure can be viewed at wileyonlinelibrary.com]

by 1°C from 2018 to 2019. There was no effect of age class, sex, or logSVL on any physiological traits (Table 2). Air temperature at time of capture (T_a) only had a significant impact on T_b (Table 2). Seven individuals (all adult males) collected in 2018 were recaptured in 2019. For this subset of resampled individuals, we found significant increases in T_{pref} (mean \pm standard error [SE]: 2018 = 31.9 ± 0.5 ; 2019 = 36.1 ± 0.4) and CT_{min} (mean \pm SE: 2018 = 4.0 ± 0.4 ; 2019 = 5.6 ± 0.6) between years, but not in T_b or CT_{max} (Table 3 and Figure 4). Although T_b and CT_{max} of individuals between years were not significantly different, the direction of the shift in mean values was positive.

4 | DISCUSSION

Across the globe 2019 was a record year for both average and maximum temperatures (NOAA, National Centers for Environmental Information, 2020), and our field site was no exception to this pattern. In comparison to 2018, central/northern Mexico was hotter in 2019 (Servicio Meteorológico Nacional, 2020). In the Mexican state of Hidalgo (where this study took place), the mean annual temperature was 18.7°C in 2018 and 19.8°C in 2019 (Servicio Meteorológico Nacional, 2020). The goal of our study was to examine whether and how thermal traits shifts across years in a natural population of a montane horned lizard, *P. orbiculare*, and consider how physiological shifts might shape their vulnerability to ongoing climate change.

Our results clearly indicate that thermal traits respond rapidly to changes in the local environment. In 2019, four key aspects of thermal physiology—body temperature, the preferred temperature, the critical thermal minimum, and the critical thermal maximum—were all significantly higher than in 2018.

Increases in heat tolerance, cold tolerance, and the preferred temperature across years hint at potentially rapid evolutionary change because the period between observations (1 year) spans the generation time for this species (Suárez-Rodríguez et al., 2018), but this possibility requires further exploration. Due to the successful recapture of individuals between years, we were able to investigate whether intragenerational plasticity was contributed to increases in thermal physiology. Even with a small recapture success between years ($n = 7$), we detected clear increases in CT_{min} and T_{pref} that are consistent in magnitude with the whole-population trends that we observed. Thus, it is possible (if not more likely) that increases in these two traits reflect, at least in large part, phenotypic plasticity in response to warmer temperatures (Bonamour, Chevin, Charmantier, & Teplitsky, 2019). Our findings echo patterns found in previous studies, which indicate that CT_{min} and T_{pref} exhibit more within-generation plasticity than CT_{max} (Clusella-Trullas & Chown, 2014; Gvoždík, 2012; Kingsolver & Huey, 1998). Thus, the ability for organisms to dynamically alter some aspects of their physiology to fluctuating environments appears to be quite strong.

Despite a population-level increase in heat tolerance, we did not observe any intragenerational shifts in heat tolerance between 2018

TABLE 2 Mixed models evaluating year, logSVL, sex, age class (categorical), and air temperature at time of capture (T_a) on thermal traits of *Phrynosoma orbiculare* (see text for details)

Trait mean \pm SE (n)	Effect	F value; p value
T_b 2018 = 31.0 ± 0.96 (28) 2019 = 33.4 ± 0.74 (26)	Year	$F_{1,48} = 5.20$; $p = .027$
	logSVL	$F_{1,48} = 0.01$; $p = .934$
	Sex	$F_{1,48} = 1.93$; $p = .171$
	Age class	$F_{1,48} = 0.17$; $p = .681$
	T_a	$F_{1,48} = 21.78$; $p < .001$
T_{pref} 2018 = 32.6 ± 0.28 (28) 2019 = 34.9 ± 0.25 (27)	Year	$F_{1,48} = 35.60$; $p < .001$
	logSVL	$F_{1,48} = 0.12$; $p = .731$
	Sex	$F_{1,48} = 0.02$; $p = .90$
	Age class	$F_{1,48} = 0.19$; $p = .664$
	T_a	$F_{1,48} = 0.08$; $p = .785$
CT_{min} 2018 = 4.38 ± 0.21 (23) 2019 = 5.62 ± 0.3 (26)	Year	$F_{1,42} = 14.64$; $p < .001$
	logSVL	$F_{1,42} = 3.89$; $p = .055$
	Sex	$F_{1,42} = 2.96$; $p = .092$
	Age class	$F_{1,42} = 1.72$; $p = .196$
	T_a	$F_{1,42} = 2.68$; $p = .109$
CT_{max} 2018 = 37.78 ± 0.23 (23) 2019 = 38.79 ± 0.27 (26)	Year	$F_{1,42} = 7.54$; $p = .009$
	logSVL	$F_{1,42} = 0.73$; $p = .396$
	Sex	$F_{1,42} = 0.25$; $p = .620$
	Age class	$F_{1,42} = 1.44$; $p = .238$
	T_a	$F_{1,42} = 0.99$; $p = .756$

Note: p -values $< .05$ are presented in bold font. Abbreviations: CT_{max} , critical thermal maximum; CT_{min} , critical thermal minimum; SE, standard error; SVL, snout-to-vent length; T_b , body temperature; T_{pref} , preferred body temperature.

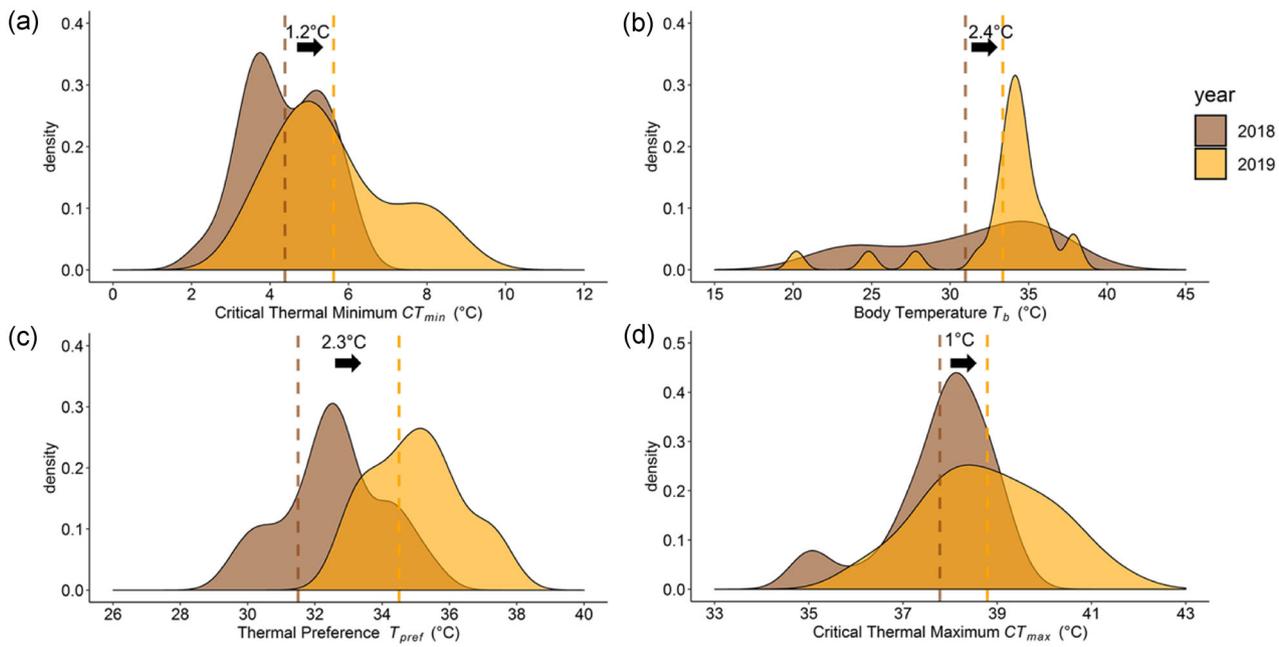


FIGURE 3 Distributions of (a) critical thermal minimum, (b) field measured body temperature, (c) thermal preference, and (d) critical thermal maximum. The thermal distributions for each variable are color coded by year, with measurements from 2018 given in brown and measurements from 2019 shown in orange. The dashed lines (also color coded by year) denote the mean of each temperature distribution. The arrows denote the direction of temperature change from 2018 to 2019, and the magnitude of the corresponding change is also given [Color figure can be viewed at wileyonlinelibrary.com]

and 2019. One possibility for this mismatch is that we lack the statistical power to detect a difference because CT_{max} increased only 1°C between years. Nonetheless, the population-level increase in CT_{min} was comparably modest (1.2°C), and an upward shift was also observed in resampled individuals. Another possibility (i.e., not necessarily mutually exclusive) is that heat tolerance exhibits a weaker long-term acclimatization response, meaning that intragenerational shifts in CT_{max} might not generally be expected on the timescale of

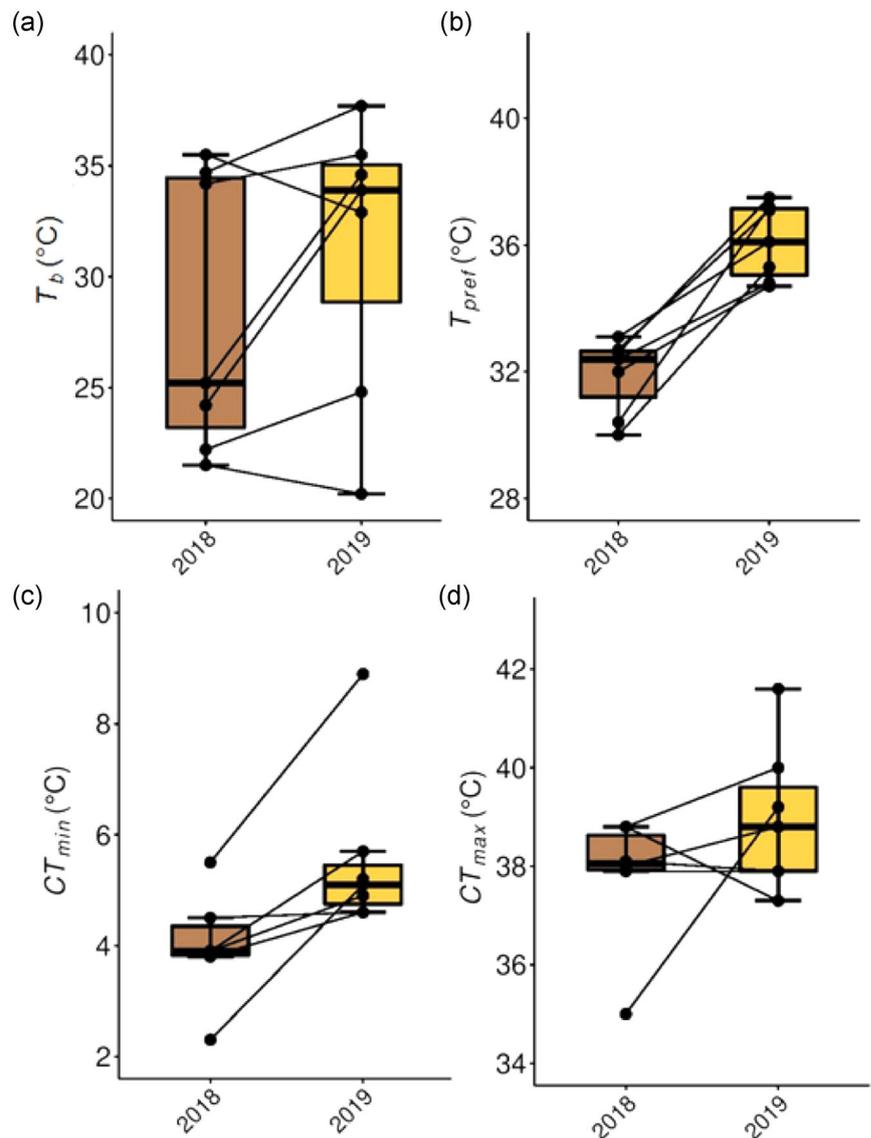
our study. When confronted with a heat stress event (such as a heat wave), lizards will typically respond with a hardening response (a rapid, transient increase in heat tolerance) that arises over a short period of time (Maness & Hutchison, 1980), such as a 6–12 hr, and persists only for a few days (Gilbert & Miles, 2019; Phillips et al., 2016). In this study, we tested for intra-individual shifts in CT_{max} on the scale of approximately a year. Thus, there is the potential that, on shorter time scales between our sampling periods,

TABLE 3 Individual repeated measures of *Phrynosoma orbiculare* of body temperature (T_b), preferred body temperature (T_{pref}), critical thermal minimum (CT_{min}), and critical thermal maximum (CT_{max}) between 2018 and 2019

ID	T_b		T_{pref}		CT_{min}		CT_{max}	
	2018	2019	2018	2019	2018	2019	2018	2019
2	25.2	34.6	30.4	37.2	5.5	8.9	37.9	37.9
5	22.2	24.8	37.6	37.5	2.3	5.1	38.8	40.0
6	21.5	20.2	32.7	37.1	3.9	5.7	38.0	38.8
12	35.5	32.9	30.0	35.3	3.9	4.9	38.1	37.9
20	34.7	37.7	32.4	34.8	3.8	4.6	38.8	37.3
22	34.2	35.5	32.0	34.7	4.5	4.6	35.0	39.2
27	24.2	33.9	33.1	36.1	NA	5.2	NA	41.6
Mean ± SE	28.2 ± 2.4	31.4 ± 2.4	31.9 ± 0.5	36.1 ± 0.4	4.0 ± 0.4	5.6 ± 0.6	37.8 ± 0.6	39.0 ± 0.6
Statistics	$t = -1.738$; $df = 6$; $p = .130$		$t = -6.953$; $df = 6$; $p = .020$		$t = -3.204$; $df = 5$; $p = .020$		$t = -0.952$; $df = 5$; $p = .385$	

Note: Results highlight the within-individual variation of thermal traits between years. p -values < .05 are presented in bold font. Abbreviations: df , degrees of freedom; SE , standard error.

FIGURE 4 Boxplot comparisons of thermal physiological traits of individual shifts between years. (a) Field measured body temperature, (b) preferred body temperature, (c) critical thermal minimum, and (d) critical thermal maximum for individuals measured in 2018 and 2019. Boxes are color coded by year with 2018 in brown and 2019 in orange. Lines connect the same individual measured in both 2018 and 2019. The dark bar represents the median, the boxes are the interquartile range, and the whiskers roughly provide the 95% confidence interval for comparing medians [Color figure can be viewed at wileyonlinelibrary.com]



individual organisms responded plastically to heat stressors, and that we missed these shifts due to the long temporal gap between our measurements. Given that we observed an increase in CT_{max} at the population level without a concomitant increase at the individual level, however, it is further possible that greater heat tolerance in 2019 represents an evolved (intergenerational) response. For example, increasing temperatures may have resulted in nonrandom survivorship and/or differential reproductive success in this population of *P. orbiculare*. Previous studies have documented that climate-induced selection on lizard physiology can be strong, often resulting in rapid phenotypic shifts over short time periods (e.g., Campbell-Staton et al., 2017; Gilbert & Miles, 2019; Leal & Gunderson, 2012; Logan, Cox, & Calsbeek, 2014), but macroevolutionary studies suggest that climatic adaptation, particularly in upper physiological limits, can be surprisingly sluggish (Bodensteiner et al., 2020; Farallo, Muñoz, Uyeda, & Miles, 2020; Salazar, Castañeda, Londoño, & Muñoz, 2019). Another possibility is that the higher heat tolerance

observed in 2019 reflects warmer maternal incubation conditions; warmer embryonic conditions in reptiles can induce overexpression of heat shock proteins that might be retained into adulthood (Gao et al., 2014). In the velvet gecko (*Amalosa lesueurii*), however, warmer embryonic conditions lead to a lower heat tolerance in hatchlings; it is unknown if these patterns persist into adulthood (Dayananda, Murray, & Webb, 2017). Another study found no effect of embryonic conditions on heat tolerance (Llewellyn et al., 2018). Thus, further work is necessary to discover the mechanistic basis for the heat tolerance shift.

On sampling days in 2019 air temperatures were 3.3°C warmer and substrate temperatures were 4.7°C warmer than on sampling days in 2018. As such, we expected a higher body temperature in field-active lizards in 2019 relative to 2018. One intriguing outcome from our study is that body temperature increased between years at the level of the whole population, but not among resampled individuals. There may be several reasons for this pattern. In 2019, the

resampled lizards were larger than the rest of the population (two-way analysis of variance: $F_{1,25} = 6.21$; $p = .02$). If larger, older lizards are able to outcompete smaller lizards for access to cooler microsites (e.g., Melville, 2002), then this might explain why resampled individuals exhibited a lower body temperature. This idea hinges on active thermoregulation by *P. orbiculare*, for which some evidence exists (Pardo-Ramirez, unpublished data), but nonetheless requires a deeper consideration using an explicit hypothesis testing framework (sensu, Hertz et al., 1993). Another possibility is that we lack the statistical power to detect a difference in body temperature among resampled individuals. Body temperature is a noisy variable: our range of observations is 17.8°C, which is more than twice as high as for T_{pref} (range = 7.9°C) and almost three times higher than for CT_{min} and CT_{max} (range = 6.6°C for both traits). With only seven resampled individuals, we suspect that we simply lacked the statistical resolution to detect differences (if they do exist). Moreover, body temperature can fluctuate substantially between days and across seasons, limiting our ability to draw robust conclusions. Despite extensive field effort, densities of *P. orbiculare* were low, which makes robust resampling of individuals (particularly for noise-prone variables) exceptionally challenging.

As global temperatures continue to rapidly rise, a question that looms large is whether organisms, especially ectotherms, will be able to keep pace with environmental change (Moritz & Agudo, 2013; Quintero & Wiens, 2013; Sinervo et al., 2010). Our general finding is that thermal traits can and do readily respond to increasing temperatures. These results are promising, as they suggest that lizards, even geographically restricted montane species like *P. orbiculare*, are equipped to tackle rapid and dramatic thermal increases in their habitat. Nonetheless, while shifts in thermal traits may confer resilience in the short term, they may not be sufficient in the long term unless warming slows or reverses (Buckley, Ehrenberger, & Angilletta, 2015; Muñoz & Moritz, 2016; Oostra, Saastamoinen, Zwaa, & Wheat, 2018; Refsnider et al., 2018). Research suggests that physiological shifts may have hard limits beyond which phenotypes become inaccessible: In the rainforest skink *Lampropholis coggeri*, for example, whether through adaptive evolution or phenotypic plasticity, the hard upper limit for locomotor performance resides near 43°C (Muñoz et al., 2016; Phillips et al., 2016). Likewise, behavioral refuges from the heat might become scarcer as environments continue to warm (Kearney et al., 2009; Kearney, 2013; Sears et al., 2016). Therefore, as organisms are confronted with progressively more hostile thermal environments, activity periods are likely to constrict, especially once phenotypic limits are reached and the costs of thermoregulation mount. If such activity constriction becomes severe, populations may decline in size or even go extinct (Basson, Levy, Angilletta, & Clusella-Trullas, 2017; Kearney, 2013; Sears et al., 2016; Sunday et al., 2014). While it is clear that plasticity can at least partially restructure the thermal phenotypes of a wild population of *P. orbiculare*, we know far less of the intrinsic limits to this ability. Moving forward, directly incorporating estimates of physiological acclimatization into dynamic energy budgets (sensu,

Riddell, Odom, Damm, & Sears, 2018) will help improve our predictions of species distribution modeling in a changing world.

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DATA AVAILABILITY STATEMENT

Data will be made available in the data repository Dryad upon manuscript acceptance.

ORCID

Brooke L. Bodensteiner  <http://orcid.org/0000-0001-6628-1923>

REFERENCES

- Angilletta, M. J. (2009). *Evolutionary Thermal Biology, Thermal Adaptation*. Oxford: Oxford University Press.
- Ballinger, R. E., Hawker, J., & Sexton, O. J. (1969). The effect of photoperiod acclimation on the thermoregulation of the lizard, *Sceloporus undulatus*. *Journal of Experimental Zoology*, 171(1), 43–47.
- Basson, C. H., Levy, O., Angilletta, M. J., Jr., & Clusella-Trullas, S. (2017). Lizards paid a greater opportunity cost to thermoregulate in a less heterogeneous environment. *Functional Ecology*, 31(4), 856–865.
- Bodensteiner, B. L., Agudelo-Cantero, G. A., Arietta, A. Z. A., Gunderson, A. R., Muñoz, M. M., Refsnider, J. M., & Gangloff, E. J. (2020). Thermal adaptation revisited: How conserved are thermal traits of reptiles and amphibians? *Journal of Experimental Zoology-A: Ecological and Integrative Physiology*. (in press, this issue).
- Bonamour, S., Chevin, L.-M., Charmantier, A., & Teplitsky, C. (2019). Phenotypic plasticity in response to climate change: The importance of cue variation. *Philosophical Transactions of the Royal Society B*, 374, 20180178.
- Buckley, L. B., Ehrenberger, J. C., & Angilletta, M. J. (2015). Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Functional Ecology*, 29, 1038–1047.
- Campbell-Staton, S. C., Cheviron, Z. A., Rochette, N., Catchen, J., Losos, J. B., & Edwards, S. V. (2017). Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard. *Science*, 357, 495–498.
- Chevin, L.-M., Lande, R., & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLOS Biology*, 8, e1000357.
- Clusella-Trullas, S., & Chown, S. L. (2014). Lizard thermal trait variation at multiple scales: A review. *Journal of Comparative Physiology B*, 184(1), 5–21.

- Dayananda, B., Murray, B. R., & Webb, J. K. (2017). Hotter nests produce hatchling lizards with lower thermal tolerance. *Journal of Experimental Biology*, 220(12), 2159–2165.
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, 105(18), 6668–6672.
- Domínguez-Guerrero, S. F., Muñoz, M. M., de Jesús Pasten-Téllez, D., Arenas-Moreno, D. M., Rodríguez-Miranda, L. A., ... Méndez-de la Cruz, F. R. (2019). Interactions between thermoregulatory behavior and physiological acclimatization in a wild lizard population. *Journal of Thermal Biology*, 79, 135–143.
- Dubey, S., & Shine, R. (2010). Restricted dispersal and genetic diversity in populations of an endangered montane lizard (*Eulamprus leuraensis*, Scincidae). *Molecular Ecology*, 19(5), 886–897.
- Dubey, S., & Shine, R. (2011). Predicting the effects of climate change on an endangered montane lizard, *Eulamprus leuraensis* (Scincidae). *Climate Change*, 107, 531–547.
- Farallo, V. R., Muñoz, M. M., Uyeda, J. C., & Miles, D. B. (2020). Scaling between macro- to microscale climatic data reveals strong phylogenetic inertia in niche evolution in plethodontid salamanders. *Evolution*, 74, 979–991.
- Fisher, M., & Muth, A. (1989). A technique for permanently marking lizards. *Herpetological Review*, 20(2), 45–46.
- Freeman, B. G., Scholer, M. N., Ruiz-Gutierrez, V., & Fitzpatrick, J. W. (2018). Climate change causes upslope shifts and mountaintop extirpations in a tropical bird community. *Proceedings of the National Academy of Sciences of the United States of America*, 115(47), 11982–11987.
- Gao, J., Zhang, W., Dang, W., Mou, Y., Gao, Y., Sun, B.-J., & Du, W.-G. (2014). Heat shock protein expression enhances heat tolerance of reptile embryos. *Proceedings of the Royal Society B*, 281, 20141135.
- Gilbert, A. L., & Miles, D. B. (2019). Antagonistic responses of exposure to sublethal temperatures: Adaptive phenotypic plasticity coincides with a reduction in organismal performance. *American Naturalist*, 194(3), 344–355.
- Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, 293(5538), 2248–2251.
- Gunderson, A. R., & Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B*, 282, 20150401.
- Gvozdík, L. (2012). Plasticity of preferred body temperatures as means of coping with climate change? *Biology Letters*, 8, 262–265.
- Hernández-Navarrete, D. (2018). *Edad y crecimiento corporal de Phrynosoma orbiculare (Squamata: Phrynosomatidae)* (Undergraduate thesis). Universidad Autónoma del Estado de México, México.
- Hertz, P. E., Huey, R. B., & Stevenson, R. D. (1993). Evaluating temperature regulation by field-active ectotherms: The fallacy of the inappropriate question. *American Naturalist*, 142(5), 796–818.
- Huey, R. B. (1982). Temperature, physiology, and the ecology of reptiles. In *Biology of the Reptilia, Physiology C: Physiological Ecology* (Vol. 12, pp. 25–91). New York, NY: Academic Press.
- Huey, R. B., Deutsch, C. A., Tewksbury, J. J., Vitt, L. J., Hertz, P. E., Álvarez Pérez, H. J., & Garland, T., Jr. (2009). Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society B: Biological Sciences*, 276(1664), 1939–1948.
- Huey, R. B., Hertz, P., & Sinervo, B. (2003). Behavioral drive versus behavioral inertia in evolution: A null model approach. *American Naturalist*, 161, 357–366.
- Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M., & Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B*, 367(1596), 1665–1679.
- Huey, R. B., Losos, J. B., & Moritz, C. (2010). Are lizards toast? *Science*, 328(5980), 832–833.
- Kearney, M. (2013). Activity restriction and the mechanistic basis for extinctions under climate warming. *Ecology Letters*, 16(12), 1470–1479.
- Kearney, M., Shine, R., Porter, W., & Wake, D. (2009). The potential for behavioral thermoregulation to buffer “cold-blooded” animals against climate warming. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 3835–3840.
- Kingsolver, J. G., & Huey, R. B. (1998). Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *American Zoologist*, 38(3), 545–560.
- Larson, M. W. (1961). The critical thermal maximum of the lizard *Sceloporus occidentalis occidentalis* Baird and Girard. *Herpetologica*, 2, 113–122.
- Leal, M., & Gunderson, A. R. (2012). Rapid change in the thermal tolerance of a tropical lizard. *American Naturalist*, 180, 815–822.
- Llewelyn, J., Macdonald, S. L., Moritz, C., Martins, F., Hatcher, A., & Phillips, B. L. (2018). Adjusting to climate: Acclimation, adaptation and developmental plasticity in physiological traits of a tropical rainforest lizard. *Integrative Zoology*, 13(4), 411–427.
- Logan, M. L., Cox, R. M., & Calsbeek, R. (2014). Natural selection on thermal performance in a novel thermal environment. *Proceedings of the National Academy of Sciences of the United States of America*, 111(39), 14165–14169.
- Maness, J. D., & Hutchison, V. H. (1980). Acute adjustment of thermal tolerance in vertebrate ectotherms following exposure to critical thermal maxima. *Journal of Thermal Biology*, 5, 225–233.
- Melville, J. (2002). Competition and character displacement in two species of scincid lizards. *Ecology Letters*, 5(3), 386–393.
- Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications*, 7, 1–14.
- Moritz, C., & Agudo, R. (2013). The future of species under climate change: Resilience or decline? *Science*, 341, 504–508.
- Muñoz, M. M., Langham, G. M., Brandley, M. C., Rosauer, D., Williams, S. E., & Moritz, C. (2016). Basking behavior predicts the evolution of heat tolerance in Australian rainforest lizards. *Evolution*, 70, 2537–2549.
- Muñoz, M. M., & Losos, J. B. (2018). Thermoregulation simultaneously promotes and forestalls evolution in a tropical lizard. *American Naturalist*, 191, E15–E26.
- Muñoz, M. M., & Moritz, C. (2016). Adaptation to a changing world: Evolutionary resilience to climate change. In J. B. Losos & R. E. Lenski (Eds.), *How evolution shapes our lives: Essays on biology and society* (pp. 238–252). Princeton, NJ: Princeton Univ. Press.
- Muñoz, M. M., Stimola, M. A., Algar, A. C., Conover, A., Rodríguez, A. J., Landestoy, M. A., ... Losos, J. B. (2014). Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. *Proceedings of the Royal Society B: Biological Sciences*, 281(1778), 20132433.
- NOAA, National Centers for Environmental Information. (2020). State of the Climate, Global Climate Report for Annual 2019. Retrieved from <https://www.ncdc.noaa.gov/sotc/global/201913>
- Oostra, V., Saastamoinen, M., Zwaa, B. J., & Wheat, C. W. (2018). Strong phenotypic plasticity limits potential for evolutionary responses to climate change. *Nature Communications*, 9, 1005.
- Paaijmans, K. P., Heinig, R. L., Seliga, R. A., Blanford, J. I., Blanford, S., Murdock, C. C., & Thomas, M. B. (2013). Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology*, 19(8), 2373–2380.
- Pardo-Ramírez, A. (2019). *Ecología térmica y horas de actividad de una población de Phrynosoma orbiculare durante su temporada de apareamiento en Hidalgo, México* (Undergraduate thesis). Universidad Juárez del Estado de Durango, México.
- Phillips, B. L., Muñoz, M. M., Hatcher, A., Macdonald, S., Llewelyn, J., Lucy, V., & Moritz, C. (2016). Heat hardening in a tropical lizard:

- Geographic variation explained by the predictability and variance in environmental temperatures. *Functional Ecology*, 30, 1161–1168.
- Pintor, A. F., Schwarzkopf, L., & Krockenberger, A. K. (2016). Extensive acclimation in ectotherms conceals interspecific variation in thermal tolerance limits. *PLOS One*, 11(3), e0150408.
- Quintero, I., & Wiens, J. J. (2013). Rates of projected climate change dramatically exceed past rates of climatic niche evolution among vertebrate species. *Ecology Letters*, 16(8), 1095–1103.
- Refsnider, J. M., Qian, S. S., Streby, H. M., Carter, S. E., Clifton, I. T., Siefker, A. D., & Vazquez, T. K. (2018). Reciprocally-transplanted lizards along an elevational gradient match thermoregulatory behavior of local lizards via phenotypic plasticity. *Functional Ecology*, 32(5), 1227–1236.
- Riddell, E. A., Odom, J. P., Damm, J. D., & Sears, M. W. (2018). Plasticity reveals hidden resistance to extinction under climate change in the global hotspot of salamander diversity. *Science Advances*, 4(7), eaar5471.
- Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., ... Wall, D. (2000). Global biodiversity scenarios for the year 2100. *Science*, 287(5459), 1770–1774.
- Salazar, J. C., Castañeda, M. R., Londoño, G. A., & Muñoz, M. M. (2019). Physiological evolution during adaptive radiation: A test of the island effect in *Anolis* lizards. *Evolution*, 73, 1241–1252.
- Sears, M. W., Angilletta, M. J., Schuler, M. S., Borchert, J., Dilliplane, K. F., Stegman, M., ... Mitchell, W. A. (2016). Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. *Proceedings of the National Academy of Sciences of the United States of America*, 113(38), 10595–10600.
- Seebacher, F. (2005). A review of thermoregulation and physiological performance in reptiles: What is the role of phenotypic flexibility? *Journal of Comparative Physiology B*, 175(7), 453–461.
- Seebacher, F., & Franklin, C. E. (2005). Physiological mechanisms of thermoregulation in reptiles: A review. *Journal of Comparative Physiology B*, 175(8), 533–541.
- Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, 5(1), 61–66.
- Servicio Meteorológico Nacional. (2020). Gobierno de México. Retrieved from <https://smn.conagua.gob.mx/es/climatologia/temperaturas-y-lluvias/resumenes-mensuales-de-temperaturas-y-lluvias>
- Sherbrooke, W. C. (2003). *Introduction to horned lizards of North America* (No. 64). Berkeley, CA: University of California Press.
- Sinervo, B., Mendez-De-La-Cruz, F., Miles, D. B., Heulin, B., Bastiaans, E., Villagrán-Santa Cruz, M., ... Sites, J. W., Jr. (2010). Erosion of lizard diversity by climate change and altered thermal niches. *Science*, 328(5980), 894–899.
- Smith, G. R., & Ballinger, R. E. (1994). Temporal and spatial variation in individual growth in the spiny lizard, *Sceloporus jarrovi*. *Copeia*, 1994(4), 1007–1013.
- Somero, G. N., Lockwood, B. L., & Tomanek, L. (2017). *Biochemical adaptation: Response to environmental challenges, from life's origins to the Anthropocene*. Sunderland, MA: Sinauer Associates, Incorporated Publishers.
- Spellerberg, I. F. (1972a). Temperature tolerances of Southeast Australian reptiles examined in relation to reptile thermoregulatory behaviour and distribution. *Oecologia*, 9(1), 23–46.
- Spellerberg, I. F. (1972b). Thermal ecology of allopatric lizards (*Sphenomorphus*) in southeast Australia. I. The environment and lizard critical temperatures. *Oecologia*, 9, 371–383.
- Sunday, J. M., Bates, A. E., Kearney, M. R., Colwell, R. K., Dulvy, N. K., Longino, J. T., & Huey, R. B. (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences of the United States of America*, 111(15), 5610–5615.
- Suárez-Rodríguez, O., Suárez-Varón, G., Chávez-Siles, F., Pérez-Arriaga, F., Andrade-Soto, G., Aguilar-Isaac, L., ... Hernández-Gallegos, O. (2018). Masa relativa de la camada en *Phrynosoma orbiculare* (Squamata: Phrynosomatidae) en el Parque Estatal Sierra Morelos, Toluca, Estado de México. *Revista Mexicana de Biodiversidad*, 89, 282–289.
- Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., ... Williams, S. E. (2004). Extinction risk from climate change. *Nature*, 427(6970), 145–148.
- Williams, J. W., Jackson, S. T., & Kutzbach, J. E. (2007). Projected distributions of novel and disappearing climates by 2100 AD. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 5738–5742.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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