

## RESEARCH ARTICLE

# The functional significance of panting as a mechanism of thermoregulation and its relationship to the critical thermal maxima in lizards

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## ABSTRACT

Because most desert-dwelling lizards rely primarily on behavioral thermoregulation for the maintenance of active body temperature, the effectiveness of panting as a thermoregulatory mechanism for evaporative cooling has not been widely explored. We measured changes in body temperature ( $T_b$ ) with increasing air temperature ( $T_a$ ) for 17 species of lizards that range across New Mexico and Arizona and quantified the temperatures associated with the onset of panting, and the capacity of individuals to depress  $T_b$  below  $T_a$  while panting, and estimated the critical thermal maxima ( $CT_{max}$ ) for each individual. We examined these variables as a function of phylogeny, body mass and local acclimatization temperature. We found that many species can depress  $T_b$  2–3°C below  $T_a$  while panting, and the capacity to do so appears to be a function of each species' ecology and thermal environment, rather than phylogeny. Panting thresholds and  $CT_{max}$  values are phylogenetically conserved within groups. Understanding the functional significance of panting and its potential importance as a thermoregulatory mechanism will improve our understanding of the potential for species' persistence in an increasingly warmer world.

**KEY WORDS:** Ectothermy, Panting, Evaporative cooling, Lizards, Thermoregulation

## INTRODUCTION

For dry-skinned ectotherms such as lizards, the mechanism and efficiency of thermoregulatory processes have significant consequences for the persistence of many species in the face of increased environmental temperatures (Sinervo et al., 2010; Sunday et al., 2014). The established view of dry-skinned ectotherm thermoregulation advocates that behavioral processes play the dominant role in the maintenance of activity body temperature ( $T_b$ ) below critical thermal limits in hot environments (Huey, 1982; Angilletta, 2009; Sunday et al., 2014; Domínguez-Guerrero et al., 2019; Muñoz and Bodensteiner, 2019) and that modifications to heat exchange through active color change and evaporative heat loss play a comparatively limited role (Stevenson, 1985). The pre-eminence of behavioral thermoregulation in lizards and the paucity of observations of thermoregulatory panting in the wild have undoubtedly supported this view (Dewitt, 1967; Judd, 1975). As a consequence, the capacity for evaporative cooling via panting in

lizards and its importance for thermoregulation have not been widely investigated (Tattersall et al., 2006).

Because lizards thermoregulate primarily by shuttling between different microclimates or by postural adjustments to maintain a preferred body temperature ( $T_{pref}$ ) before initiating open-mouthed panting, the onset of panting has historically been viewed as an emergent response to unavoidable heat exposure and of approaching lethal  $T_b$  values near the animal's critical thermal maximum ( $CT_{max}$ ; Weese, 1917; Cowles and Bogert, 1944; Dawson and Templeton, 1966; Vernon and Heatwole, 1970; Webb et al., 1972; Tattersall et al., 2006). Lizards were traditionally assumed to avoid panting during their normal daily activity because either maximum rates of evaporation during panting were deemed insufficient for dissipating environmental heat loads or incurred costs would produce substantial water deficits (Crawford and Kampe, 1971; Dawson and Templeton, 1963; Mayhew, 1968; Mautz, 1982). These studies concluded that the inherent cooling capacities of most species are modest and provide only a limited delay in the onset of lethal hyperthermia with increasing environmental heat loads (Dawson, 1960; Templeton, 1960; Dawson and Templeton, 1963, 1966).

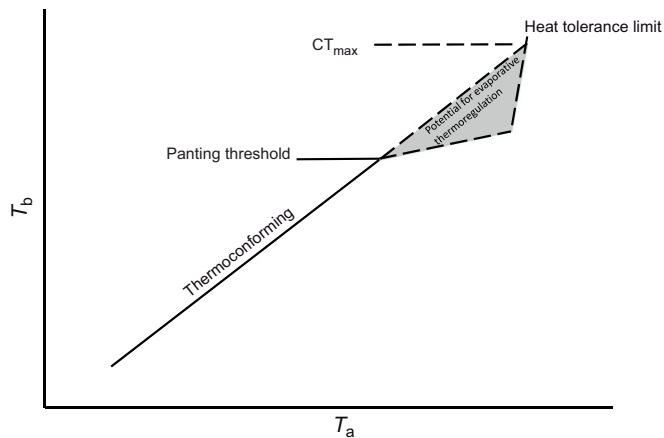
Evaporative cooling has the potential to lower the operative temperature [ $T_e$ ; the equivalent  $T_b$  that incorporates the effects of air temperature ( $T_a$ ), wind speed and radiative exchange] of lizards, thus potentially allowing animals to remain active while maintaining a thermal safety margin equivalent to retreating to a cooler micro-habitat (Fig. 1; Sunday et al., 2014). Stevenson (1985) estimated that panting may produce an air–body temperature difference of up to 5°C, potentially having utility as a thermoregulatory mechanism for extending activity in hot environments. Indeed, for certain species, panting appears to be an effective strategy for lowering head temperature below body temperature in hot conditions (Webb et al., 1972; Case, 1972; Crawford et al., 1977). Furthermore, passive evaporation via other mucous membranes (e.g. the eyes or cloaca) has also been demonstrated as a route for evaporative heat loss (Heath, 1964; Firth, 1979; DeNardo et al., 2004). Although evaporative water loss associated with active panting may be costly to an animal's water balance, it may provide other benefits such as allowing the prolonged defense of territories (Dewitt, 1967; Judd, 1975), continued foraging or the avoidance of predators (Dewitt, 1967; Christian and Tracy, 1981; Grant and Dunham, 1988). Consequently, species that have sufficient water reserves to cope with, or even exploit environments where  $T_e$  is above the preferred values may thus be well suited to expand their thermal niche and increase their fitness (Tracy and Christian, 1986; Kearney and Porter, 2004).

In addition to the role of panting as a thermoregulatory strategy, the onset of panting in relation to a lizard's voluntary thermal limits and the  $CT_{max}$  is important to consider, as it may provide a sublethal

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**Fig. 1. Conceptual diagram of shifts in body temperature under increasing environmental heat following panting.** The gray area represents the potential range of body temperatures ( $T_b$ ) a lizard could maintain following the onset of panting up until they reach their critical thermal maximum ( $CT_{max}$ ).

benchmark of a lizard's thermoregulatory status in relation to lethal thermal limits. Whether the onset of panting is indicative of the proximity to the  $CT_{max}$  is largely contextual, depending on individual condition and environmental heat loads. Camacho et al. (2018), for example, found lizards panting at or near their voluntary thermal maximum  $T_b$ ; panting was labile and depended on exposure time and temperature. Hydration, ecology or acclimation history (Chong et al., 1973; Heatwole et al., 1975; Parmenter and Heatwole, 1975; Dupré and Crawford, 1985; da Silveria Scarpellini et al., 2015) may also determine whether individuals employ panting as a thermoregulatory strategy. Body size and age may also mediate the use of panting as small-bodied lizards are subject to higher mass-specific heat and water fluxes compared with large-bodied lizards, producing differing costs and benefits for lizards of different size classes (Mautz, 1982; Toledo et al., 2008).

The proximity of the panting threshold to the  $CT_{max}$  may be dependent on the functional niche a lizard occupies, as well as its evolutionary history. Species that are adapted to hot, arid environments tend to have higher thermal tolerances and/or evaporative capacities, and may have a greater capacity for evaporative cooling when exposed to high  $T_a$  or large solar heat loads (Claussen, 1967; Case, 1972; Brusch et al., 2015). Additionally, foraging mode likely interplays with the thermoregulatory tradeoffs associated with panting. For example, lizards that are sit-and-wait foragers in exposed habitats, such as the genus *Phrynosoma*, commence panting approximately 5°C before reaching their  $CT_{max}$  (Kour and Hutchison, 1970), and may employ panting to extend their time foraging, whereas lizards that are active foragers, such as the genus *Aspidoscelis*, will more likely rely on thermal shuttling between thermal environments over evaporative cooling to modulate their  $T_b$  (Ryan et al., 2016). Given that the  $T_b$  associated with panting may be variable and influenced by a variety of factors, it is unknown whether panting threshold, and the capacity to cool evaporatively, is a conserved trait among lizard groups or whether it is phylogenetically independent.

In this investigation, we examined the capacity of lizards to actively thermoregulate by depressing their body temperature via panting while exposed to prolonged, increasing heat loads. We continuously measured  $T_a$  and  $T_b$  prior to and following the onset of panting up until the  $CT_{max}$  in 17 lizard species from the American southwest. We asked the following questions. (1) Does panting

threshold provide a strong indicator of critical thermal limits ( $CT_{max}$ )? (2) When exposed to high  $T_a$ , can lizards maintain  $T_b$  below  $T_a$  by panting and how does this capacity vary among species? (3) Are panting threshold,  $CT_{max}$  and  $T_b$  depression capacity phylogenetically conserved? (4) Does body size or environmental acclimatization influence panting threshold,  $CT_{max}$  or cooling capacity? (5) How might the capacity for  $T_b$  depression allow for extended activity periods under intense heat scenarios? Because panting threshold can be influenced by a variety of factors, we hypothesized that there would not be a significant relationship between panting threshold and  $CT_{max}$ . Additionally, because different ecological guilds may be subject to different thermal environments, we hypothesized the ability to maintain a greater  $T_a$ – $T_b$  gradient would result from species' adaptation to their respective habitats and ecology, rather than their evolutionary history.

## MATERIALS AND METHODS

### Study species and handling procedures

A total of 262 lizards, representing 13 species from Phrynosomatidae: zebra-tailed lizard (*Callisaurus draconoides* Blainville 1835), greater earless lizard (*Cophosaurus texanus* Troschel 1852), Texas horned lizard [*Phrynosoma cornutum* (Harlan 1825)], greater short-horned lizard (*Phrynosoma hernandesi* Girard 1858), round-tailed horned lizard (*Phrynosoma modestum* Girard 1852), regal horned lizard (*Phrynosoma solare* Gray 1845), twin-spotted spiny lizard (*Sceloporus bimaculosus* Phelan and Brattstrom 1955), Clark's spiny lizard (*Sceloporus clarkii* Baird and Girard 1852), southwestern fence lizard (*Sceloporus cowlesi* Lowe and Norris 1956), Yarrow's spiny lizard (*Sceloporus jarrovi* Cope 1875), crevice spiny lizard (*Sceloporus poinsettii* Baird and Girard 1852), ornate tree lizard [*Urosaurus ornatus* (Baird and Girard 1852)], side-blotched lizard (*Uta stansburiana* Baird and Girard 1852); one species from Crotophytidae: eastern collared lizard [*Crotaphytus collaris* (Say 1822)]; one species from Teiidae: Chihuahuan spotted whiptail [*Aspidoscelis exsanguis* (Lowe 1956)]; and two species from Iguanidae: desert iguana [*Dipsosaurus dorsalis* (Baird and Girard 1852)], chuckwalla (*Sauromalus ater* Duméril 1856) were used for this study (Table 1). Lizards were captured at multiple localities in New Mexico and Arizona between May and September of 2016, 2017 and 2018, and in May of 2019. The *S. ater* were captive-hatched individuals from parents originating in Riverside Co., CA, USA, and were kept in semi-naturalistic outdoor enclosures prior to trials.

Animals were captured with a lasso attached to a 12 foot (~3.7 m) long pole or by hand. Following capture, lizards were held in cloth bags and transported to the University of New Mexico laboratory in a cooler that was kept in a climate-controlled environment (room temperature or cooler) to reduce activity. Animals were held no longer than 72 h prior to trials, with the majority of individuals (>60%) undergoing a trial within 24 h of being captured. Lizards held longer than 48 h were temporarily housed in terraria with food and heat lighting available. Although all species used in these trials obtain their water from their diet, we also offered animals water *ad libitum*, by placing either a wet paper towel or free-standing water in their cage. Following trials, lizards were euthanized via injection with a mixture of MS-222 and water and deposited in the Museum of Southwestern Biology. Animal care protocols were approved by the Institutional Animal Care and Use Committee of the University of New Mexico (protocol no. 16-200437-MC). Lizards were captured under permits from the New Mexico Game and Fish

Table 1. Thermal parameters for the 17 study species

Species	N	M <sub>b</sub> (g)	PT (°C)	PT range (°C)	CT <sub>max</sub> (°C)	CT <sub>max</sub> range (°C)	HTL (°C)	PT–CT <sub>max</sub> differential (°C)	Max. T <sub>a</sub> –T <sub>b</sub> gradient (°C)	T <sub>a</sub> –T <sub>b</sub> slope		
										Before panting	After panting	Mass–PT slope
<b>Teiidae</b>												
<i>Aspidoscelis exsanguis</i>	17	16.6±1.1	43.7±0.3	39.8–45.7	45.6±0.2	44.2–46.8	46.1±0.4	2.0±0.3	0.7±0.1	0.97	0.79	−0.17 (−0.29, −0.06)*
<b>Crotaphytidae</b>												
<i>Crotaphytus collaris</i>	28	31.2±1.6	41.5±0.2	40.1–43.5	45.4±0.2	43.7–46.6	48.4±0.1	4.2±0.1	2.7±0.2	0.89	0.64*	−0.39 (−0.65, −0.06)*
<b>Iguanidae</b>												
<i>Dipsosaurus dorsalis</i>	12	36.1±4.0	43.4±0.4	41.4–45.3	47.4±0.2	46.6–48.9	49.1±0.2	4.0±0.4	1.9±0.2	0.96	0.68*	−0.06 (−0.11, −0.01)*
<i>Sauromalus ater</i>	9	114.2±8.9	42.5±0.3	40.2–43.5	46.6±0.2	45.6–47.1	48.0±0.1	4.1±0.4	1.7±0.2	0.90	0.67*	−0.03 (−0.05, −0.01)*
<b>Phrynosomatidae</b>												
<i>Callisaurus draconoides</i>	10	12.3±0.8	44.3±0.3	42.6–45.9	46.3±0.1	45.7–47.2	48.2±0.2	2.0±0.3	1.5±0.2	0.99	0.68*	0.20 (−0.03, 0.44)
<i>Cophosaurus texanus</i>	19	10.0±0.7	43.2±0.2	41.5–45.0	45.5±0.1	44.5–46.6	47.4±0.2	2.9±0.2	1.4±0.3	0.96	0.62*	−0.09 (−0.22, 0.04)
<i>Phrynosoma cornutum</i>	5	33.9±4.5	40.5±0.7	38.4–41.5	44.8±0.1	44.6–46.2	48.1±0.2	4.7±0.7	2.5±0.3	0.90	0.68*	0.11 (0.01, 0.21)
<i>Phrynosoma hernandesi</i>	17	21.9±4.6	40.2±0.3	37.8–42.2	45.4±0.1	44.5–46.6	47.5±0.2	5.5±0.3	2.3±0.2	0.89	0.72*	−0.24 (−0.51, 0.03)*
<i>Phrynosoma modestum</i>	8	6.5±2.0	41.9±0.8	41.1–42.9	45.9±0.1	44.7–46.5	48.6±0.3	3.5±0.2	2.7±0.2	0.96	0.54*	−0.19 (−0.036, −0.03)*
<i>Phrynosoma solare</i>	2	34.6±9.2	41.0±0.8	40.2–41.9	45.7±0.6	45.1–45.5	47.5±0.8	4.3±1.1	2.5±0.5	0.92	0.57*	NA
<i>Sceloporus bimaculosus</i>	12	29.4±6.3	39.9±0.3	38.2–41.2	44.5±0.1	43.2–45.2	45.8±0.2	4.5±0.3	1.8±0.2	0.89	0.76*	−0.02 (−0.04, 0.01)
<i>Sceloporus clarkii</i>	24	23.8±2.3	40.2±0.3	37.8–41.8	44.0±0.2	40.0–45.2	46.0±0.2	4.5±0.3	2.3±0.2	0.95	0.64*	−0.17 (−0.05, −0.001)*
<i>Sceloporus cowlesi</i>	26	8.2±0.5	39.6±0.2	37.3–41.7	43.5±0.2	41.6–45.6	44.5±0.2	3.7±0.2	1.6±0.2	0.91	0.73*	−0.16 (−0.33, 0.01)*
<i>Sceloporus jarrovi</i>	11	14.4±2.4	39.3±0.3	37.6–40.4	43.5±0.2	42.0–44.3	44.2±0.2	4.2±0.3	1.0±0.1	0.91	0.82	0.03 (−0.04, 0.11)
<i>Sceloporus poinsettii</i>	28	29.9±3.6	39.1±0.2	36.8–41.6	43.6±0.1	41.6–44.5	44.3±0.3	4.7±0.2	0.9±0.1	0.94	0.89	−0.08 (−0.16, −0.01)*
<i>Urosaurus ornatus</i>	22	3.4±0.2	42.9±0.3	41.1–45.3	45.0±0.6	43.5–46.0	46.6±0.3	2.3±0.3	1.7±0.3	0.92	0.69*	−0.48 (−1.54, 0.58)
<i>Uta stansburiana</i>	10	3.3±0.3	42.4±0.6	41.0–43.7	43.8±0.9	43.4–45.5	45.0±0.6	1.2±0.2	1.5±0.3	0.86	0.67*	−0.58 (−2.63, 1.48)

Mean (±s.e.) body temperature ( $T_b$ ) thermal thresholds for the lizard species measured, thermal threshold range, panting threshold range, panting threshold–critical thermal maximum differential (PT–CT<sub>max</sub>), maximum air temperature ( $T_a$ )– $T_b$  gradient maintained while panting,  $T_a$ – $T_b$  slopes during experimental trials, and slope of the relationship between body mass ( $M_b$ ) and panting threshold (PT) for each species, with 95% confidence interval (CI; slope, lower 95% CI, upper 95% CI). HTL, heat tolerance limit.

\* $p < 0.05$ .

Department (#3627) and the Arizona Game and Fish Department (#SP510878). Ironwood Forest National Monument also granted permission to collect animals.

### **$T_a$ and $T_b$ measurements**

$T_b$  measurements were made over a  $T_a$  range of 35 to 50°C using a flow-through respirometry system, where incurrent air was scrubbed of CO<sub>2</sub> and H<sub>2</sub>O using a purge gas generator (model PCRMBX1A##-F, Puregas LLC, Broomfield, CO, USA), which produces air with a dew point of <−20°C. The respirometry chamber consisted of a transparent plastic container (1.7 l, 12 cm×8 cm×16 cm for lizards under 50 g; 3.6 l, 20 cm×8 cm×22 cm for lizards over 50 g; Snapware Total Solutions, Pyrex, Greencastle, PA, USA) sealed by a snap-latch lid lined with a gasket. Dry, CO<sub>2</sub>-free air was pushed through the respirometry chamber at flow rates of 0.5–5 standard liters per minute (SLM) with a digital mass-flow controller (MC-Series, Alicat, Tucson, AZ, USA) that ensured chamber dew points did not exceed 5°C (measured with a CO<sub>2</sub>/H<sub>2</sub>O gas analyzer; model LI-840A in 2017, model LI-7000 in 2018 and 2019; LI-COR, Lincoln, NE, USA). The lizard chamber was placed inside an environmental chamber (model no. 166VL, Percival Scientific, Perry, IA, USA) where temperature was controlled to ±0.5°C.  $T_a$  and  $T_b$  were measured using a thermocouple thermometer (model TC-2000, Sable Systems, Las Vegas, NV, USA) with two Cu-Cn thermocouples (model RET-4, Physitemp, Clifton, NJ, USA) inserted into each chamber via a small hole sealed with silicone. One thermocouple measured chamber  $T_a$  and the second thermocouple was inserted ~10 mm (up to 20 mm for larger lizards such as chuckwallas) into the lizard's cloaca to measure  $T_b$ , and was held in place with a 1 cm wide piece of vinyl electrical tape.

### **Experimental protocol**

Lizards were weighed to ±0.1 g accuracy on a digital scale (model V31XH202, Ohaus, Parsippany, NJ, USA) and placed into the chamber at a  $T_a$  of 35°C and were left until  $T_a$  and  $T_b$  equilibrated (~30 min), which allowed for habituation to the chamber environment. Any fecal material produced prior to or during the trial was removed from the chamber, weighed and subtracted from initial body mass, and the chamber environment was allowed to re-equilibrate. Experimental trials commenced when  $T_b$  was equal to  $T_a$  (±0.2°C). For each trial, we started all lizards at 35°C and then increased  $T_a$  to 38°C, followed by increases in  $T_a$  in 2°C increments until the lizard reached its CT<sub>max</sub>. Lizards were held at each temperature for approximately 30 min before ramping up to the next temperature. Trials on average lasted 4.0 h and animals lost an average of 6.4% of body mass ( $M_b$ ) during the trial period.

Two video cameras were placed in the environmental chamber with the lights on in front of each chamber, so lizard activity could be monitored continuously. Lizard activity during measurements was categorized as 'inactive', 'brief activity', 'continuous activity' and 'panting'. It was noted when lizards were continuously active, when a lizard engaged in running or jumping for longer than 5 s, and those measurements were removed from the data series, though lizards were typically less active at higher  $T_a$  and/or when they were panting. As  $T_a$  increased,  $T_a$  and  $T_b$  were recorded when a lizard commenced continuous open-mouth panting. Because  $T_b$  typically fell below  $T_a$  following the onset of panting, a trial was immediately ended when: (1) there was a sharp increase in  $T_b$ , indicating evaporative cooling was no longer effective, (2) a lizard showed prolonged distress or escape behavior (i.e. continuous running/jumping) for >5 min, (3) chamber CO<sub>2</sub> values fell sharply, indicating heat shock, and (4) a lizard showed a loss of balance or

righting response (LRR), including the onset of spasms (OS; Lutterschmidt and Hutchison, 1997), although we did not physically test righting response by rolling lizards on their backs. Because CT<sub>max</sub> is regarded as the  $T_b$  at which an animal loses its ability to functionally thermoregulate to avoid lethal conditions (Cowles and Bogert, 1944; Lutterschmidt and Hutchison, 1997), we designated the  $T_b$  at the end of the trial as the animal's CT<sub>max</sub>. To avoid high *in situ* mortality and unnecessarily replicate previous estimates that would have been lethal to individuals, in addition to our need to maintain an airtight seal on the chambers, our estimates of CT<sub>max</sub> may be more conservative than those in other studies. Nevertheless, the apparent inability of lizards to maintain balance, combined with the rapid increase in  $T_b$ , suggested that lizards had either reached or were very close to reaching their CT<sub>max</sub>, and generally agree with previous estimates for a number of species (see below). Following each trial, the lizard was removed from the chamber, reweighed and allowed to recover in a cotton bag with a wet paper towel until its  $T_b$  stabilized, prior to injection of MS-222 as above.

### **Data analysis**

All analyses were carried out using R software (version 3.4.3, <http://www.R-project.org/>). In order to detect a phylogenetic signal for panting threshold ( $T_b$ ), CT<sub>max</sub> ( $T_b$ ) and maximum  $T_a$ – $T_b$  gradient, we applied Blomberg's *K* and Pagel's  $\lambda$  to a phylogeny adapted from Pyron et al. (2013) and pruned to only include taxa for which we collected data. Statistics and tree manipulations were done using the *phytools* (<https://CRAN.R-project.org/package=phytools>) and *ape* (<https://CRAN.R-project.org/package=ape>) packages, respectively. We derived phylogenetic independent contrasts (PIC) to remove effects of phylogeny prior to running regressions comparing  $M_b$  (g), panting threshold, CT<sub>max</sub>, maximum  $T_a$ – $T_b$  gradient and panting threshold–CT<sub>max</sub> proximity. Some taxonomic naming discrepancies exist between the Pyron et al. (2013) phylogeny and our dataset. In such cases, we retained tips of the phylogeny that represent the species complex to which our studied taxa belong (e.g. *S. bimaculosus* is a member of the species complex represented by *Sceloporus magister* in Pyron et al., 2013). We manually updated taxa names in the trees presented to simplify comparison of phylogenies and results for *S. bimaculosus*, *S. cowlesi* on the *S. undulatus* branch, and *A. exsanguis* on the *Aspidoscelis sexlineatus* branch.

To account for the effect of thermal acclimatization on the thermoregulatory capacity of lizards, we extrapolated mean daily maximum temperature for the 30 day period prior to lizard capture at the capture site (PRISM climate group, Oregon State University). We used a linear mixed-effects model when analyzing lizard panting threshold, CT<sub>max</sub> and maximum  $T_a$ – $T_b$  gradient maintained with the *nlme* package in R (<https://CRAN.R-project.org/package=nlme>), with lizard species,  $M_b$  and acclimatization temperature treated as main effects and individual lizards treated as a random factor. We used likelihood ratio tests and AIC scoring in model selection where the models compared tested for interactions of main effects. Because the *S. ater* originated from a captive colony, and therefore were not necessarily acclimated to wild-caught conditions, we did not include them in the model analyses concerning acclimatization temperature. To determine effect of panting on  $T_b$ , panting status was divided into two categories: 'not panting' when no panting was observed and 'panting' when continuous panting was observed. Because lizard  $T_b$  increases with  $T_a$ , we included  $T_a$  as a covariate in ANCOVA to examine the effect of panting on  $T_b$  for each species. Slopes of lines were compared using the *lsmeans* package in R (<https://cran.r-project.org/package=lsmeans>).



## RESULTS

 $T_b$ 

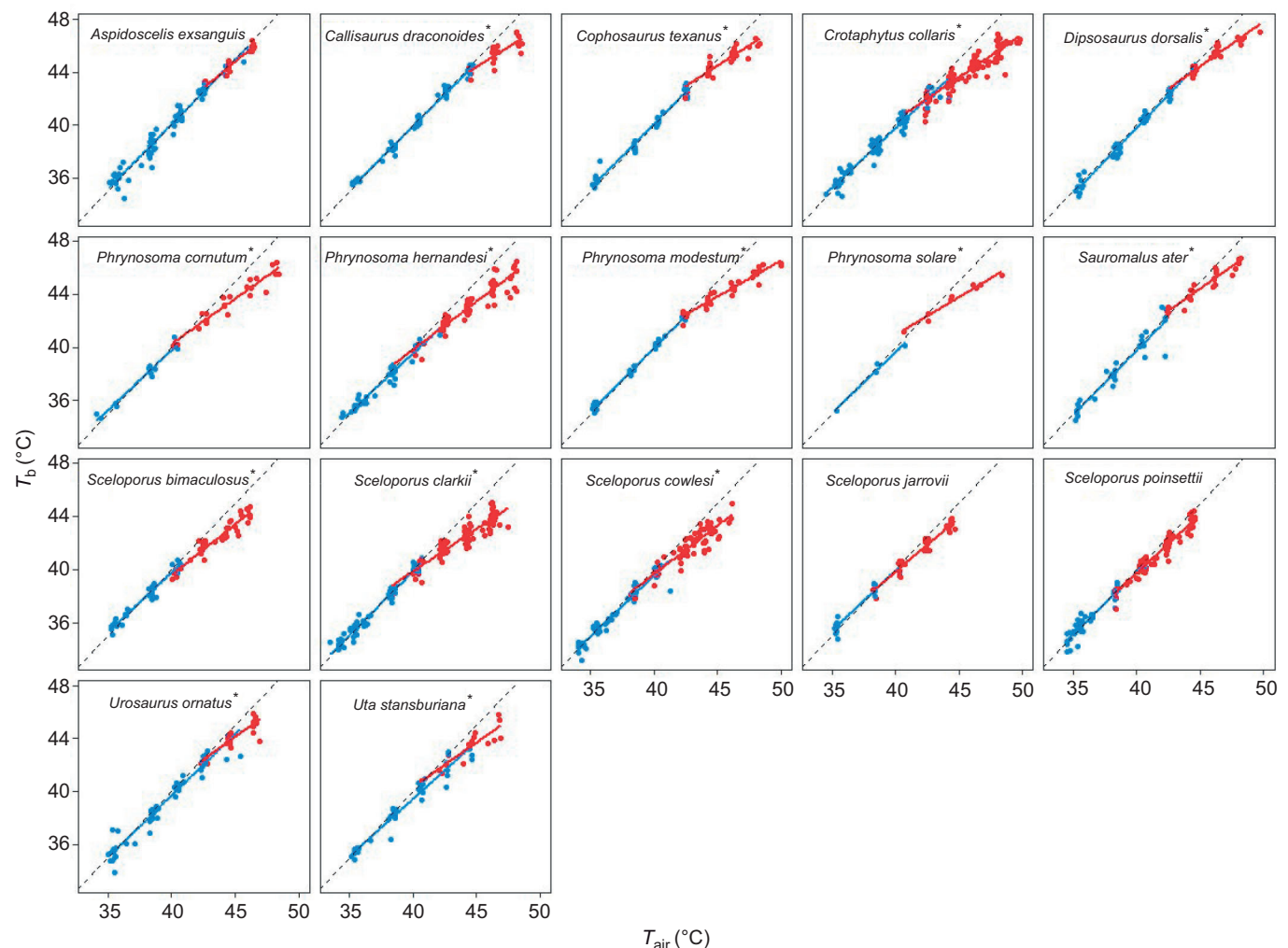
Panting caused the  $T_b$  slope to significantly deviate from the pre-panting slope for 14 species from three families (Fig. 2): *C. collaris* ( $F_{1,183}=51.4$ ,  $P<0.001$ ), *P. hernandesi* ( $F_{1,109}=11.7$ ,  $P<0.01$ ), *P. cornutum* ( $F_{1,31}=7.9$ ,  $P<0.01$ ), *P. modestum* ( $F_{1,54}=143.1$ ,  $P<0.01$ ), *P. solare* ( $F_{1,9}=13.7$ ,  $P<0.01$ ), *C. draconoides* ( $F_{1,63}=25.5$ ,  $P=0.01$ ), *C. texanus* ( $F_{1,120}=56.9$ ,  $P<0.001$ ), *D. dorsalis* ( $F_{1,73}=50.6$ ,  $P<0.01$ ), *S. ater* ( $F_{1,57}=17.6$ ,  $P<0.01$ ), *S. bimaculosus* ( $F_{1,65}=5.6$ ,  $P=0.02$ ), *S. clarkii* ( $F_{1,144}=63.2$ ,  $P<0.01$ ), *S. cowlesi* ( $F_{1,117}=12.3$ ,  $P<0.01$ ), *U. ornatus* ( $F_{1,86}=9.3$ ,  $P<0.01$ ) and *U. stansburiana* ( $F_{1,49}=4.5$ ,  $P=0.038$ ).  $M_b$  had no significant effect on the slope of  $T_b$  while panting for any species. However,  $M_b$  within a species significantly predicted panting threshold; we thus included mass as a covariate in subsequent models predicting panting threshold.

The largest gradients between  $T_a$  and  $T_b$  were observed in *C. collaris*, with an average value of  $2.7\pm0.8^\circ\text{C}$  and a maximum gradient observed of  $4.79^\circ\text{C}$  in a large male (mass 30.92 g). Among Phrynosomatid lizards, there was high variability in their ability to reduce  $T_b$  below  $T_a$  while panting. All species of *Phrynosoma* also maintained large  $T_a-T_b$  gradients, with *P. modestum* having a mean maximum gradient of  $2.7\pm0.6^\circ\text{C}$ . Species in the genus *Sceloporus*

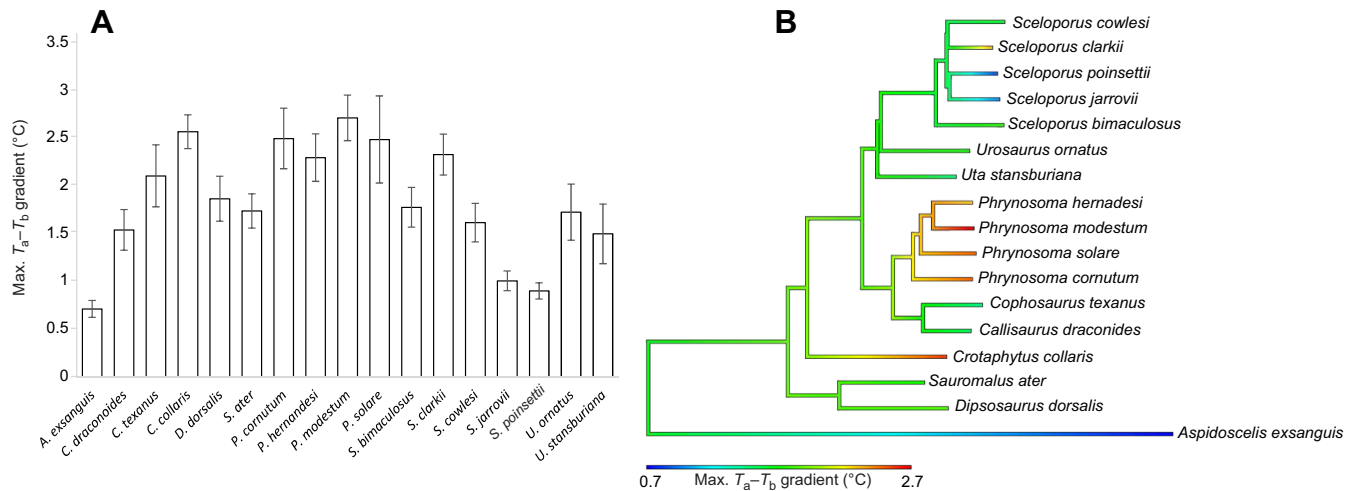
showed greater variability in the capacity to cool and the size of the  $T_a-T_b$  gradient; *S. bimaculosus*, *S. clarkii* and *S. cowlesi* showed  $T_a-T_b$  differentials of  $2-3^\circ\text{C}$ , with *S. clarkii* notably reducing its  $T_b$  by as much as  $3.5^\circ\text{C}$  while panting (Table 1). In contrast, *S. jarrovi* and *S. poiretii* were able to establish only modest  $T_a-T_b$  gradients not exceeding  $1.5$  and  $1.3^\circ\text{C}$ , respectively (Fig. 3A). Among smaller bodied species such as *U. ornatus* and *U. stansburiana*, we found high variability in the capacity to lower  $T_b$  while panting, with gradients ranging from  $>3^\circ\text{C}$  to  $<1^\circ\text{C}$  for the two species, respectively.

Panting threshold and  $CT_{\max}$ 

The  $T_b$  at which the onset of panting occurred was highly variable among species and tended to be positively correlated with acclimatization temperature and negatively correlated with  $M_b$  (see below). There was a positive, albeit non-significant relationship between panting threshold and  $CT_{\max}$  ( $F_{1,14}=3.17$ ,  $P=0.09$ ). Most species began panting at a  $T_b$  of  $\sim 3-4^\circ\text{C}$  below their respective  $CT_{\max}$ , with only *A. exsanguis*, *U. stansburiana* and *C. draconoides* refraining from panting until their  $T_b$  was  $<2^\circ\text{C}$  below their  $CT_{\max}$  (Fig. 4A). There was no significant effect of  $M_b$  on the maximum  $T_a-T_b$  gradient observed while panting ( $F_{1,14}=0.73$ ,  $P=0.40$ ) and a non-significant positive association between the panting



**Fig. 2.**  $T_b$  as a function of air temperature ( $T_a$ ). Blue lines indicate  $T_b$  before panting. Red lines indicate  $T_b$  after panting. Dashed lines represent  $T_b=T_a$ . Asterisks indicate species whose panting slope significantly deviated from the  $T_a=T_b$  line.



**Fig. 3.  $T_a - T_b$  gradient.** (A) Mean  $\pm$  s.e. maximum  $T_a - T_b$  gradient maintained while panting. (B) Phylogeny of maximum  $T_a - T_b$  gradient for the species sampled.

threshold- $CT_{max}$  proximity and maximum  $T_a - T_b$  gradient maintained across species ( $F_{1,14}=0.56$ ,  $P=0.49$ ).

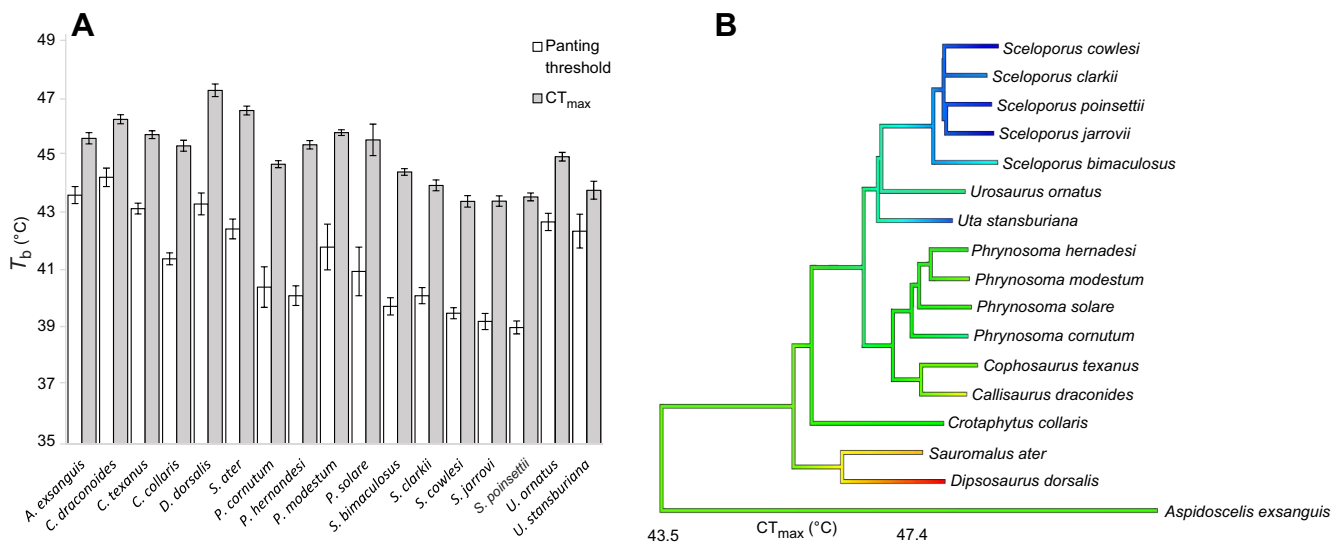
In models where lizard species was not included as a factor, there was a significant effect of  $M_b$  ( $F_{1,231}=30.18$ ,  $P<0.001$ ) on panting threshold, and an effect of maximum acclimatization temperature on panting threshold ( $F_{1,231}=84.73$ ,  $P<0.001$ ),  $CT_{max}$  ( $F_{1,230}=86.1$ ,  $P<0.001$ ) and maximum  $T_a - T_b$  gradient ( $F_{1,230}=5.12$ ,  $P=0.02$ ). Because panting threshold for each species varied with  $M_b$  (species  $\times$  mass,  $F_{15,202}=1.9$ ,  $P=0.03$ ), but not acclimatization temperature [species  $\times$  maximum temperature ( $T_{max}$ ),  $F_{15,202}=0.96$ ,  $P=0.50$ ] in models where species was included as a factor, we evaluated the panting threshold-mass relationships for each species independently with a linear mixed effects model and report parameter estimates with 95% confidence intervals (CI) in Table 1. There was no significant effect between  $M_b$  and maximum acclimatization temperature (mass  $\times T_{max}$ ,  $F_{1,230}=0.8$ ,  $P=0.37$ ) when predicting  $CT_{max}$ .

### Phylogenetic signal

We determined that there was no significant phylogenetic signal for maximum  $T_a - T_b$  gradient maintained while panting ( $K=0.59$ ,  $P=0.06$ ,  $\lambda=0.68$ ,  $P=1$ ; Fig. 3B). However, there were strong phylogenetic signals for panting threshold ( $K=1.16$ ,  $P=0.01$ ,  $\lambda=0.99$ ,  $P=0.02$ ) and for  $CT_{max}$  ( $K=1.54$ ,  $P<0.001$ ,  $\lambda=0.99$ ,  $P<0.001$ ; Fig. 4B). After correcting for phylogeny, we determined there was no significant relationship between  $M_b$  and panting threshold ( $F_{1,14}=1.85$ ,  $P=0.19$ ,  $T_b$ ) or  $CT_{max}$  ( $F_{1,14}=0.43$ ,  $P=0.51$ ) across species.

### DISCUSSION

Our results show that for 14 of 17 lizard species, open-mouth panting is an effective mechanism to lower  $T_b$  below  $T_a$  when animals are exposed to high environmental heat loads. We found that the ability to evaporatively cool varied greatly among species; for example, the large-bodied sit-and-wait predator *C. collaris* was able to maintain a  $T_b$  as much as 4.8°C below  $T_a$ . In contrast, the



**Fig. 4. Panting threshold and  $CT_{max}$ .** (A) Mean  $\pm$  s.e. panting threshold ( $T_b$ ) and  $CT_{max}$  ( $T_b$ ) of lizards sampled. Most species began panting 3–5°C below  $CT_{max}$ . (B) Phylogeny of  $CT_{max}$  for the species sampled.  $CT_{max}$  is strongly conserved within groups; *Sceloporus* all have similarly low  $CT_{max}$  values, whereas *Phrynosoma*  $CT_{max}$  values are consistently moderate and Iguanid values are consistently high.

active forager *A. exsanguis* was unable to significantly reduce its  $T_b$  below  $T_a$  via panting. These results expand our view of the potential importance of panting as a thermoregulatory mechanism that was not fully appreciated by early studies (Dawson and Templeton, 1963, 1966; Mautz, 1982; but see Case, 1972; Dewitt, 1967). We found that species from cooler habitats had lower  $CT_{max}$  and panting thresholds. We also found  $M_b$  had no effect on interspecific differences in  $CT_{max}$  and panting threshold but did influence intraspecific variability in panting thresholds. Our results show that the  $T_b$  at the onset of panting was variable and provides a modest but not definitive ability to predict estimated  $CT_{max}$ . In the following paragraphs, we examine how factors such as thermal acclimatization, phylogeny and body size affect cooling ability, and how consideration of cooling capacity may influence our understanding of the fates of lizard communities under future climates.

### Effects of habitat differences on cooling capacity

The high variability in the maximum  $T_a$ – $T_b$  gradients observed across the species tested, especially within Phrynosomatid lizards, suggests differences in cooling capacity result from thermoregulatory pressures associated with various habitat characteristics such as elevation and  $T_a$ . For example, *S. jarrovi* and *S. poinsettii* inhabit mid- to high-elevation environments in Arizona and New Mexico where environmental heat loads are not as great as in lowland habitats. *Sceloporus jarrovi* and *S. poinsettii* typically rely on shuttling to thermal retreats such as rocks and crevices (Middendorf and Simon, 1988; Degenhardt et al., 1996) and show a very limited ability to lower their  $T_b$  via panting or tolerate high heat loads. In contrast, species from hotter habitats such as *S. clarkii* and *S. bimaculosus* are notably more capable of lowering their  $T_b$  via panting. The desert obligate *S. bimaculosus* was able to reduce  $T_b$  below  $T_a$  by 1.8°C, while the widespread *S. clarkii* was able to reduce  $T_b$  below  $T_a$  by an average of 2.3°C, reflecting their higher environmental heat loads (Figs 2 and 3). The average  $T_b$  depression of 1.6°C for *S. cowlesi* indicates a more modest cooling ability, although like *S. clarkii*, this species is also found across a wide range of elevations and habitat types. Although panting threshold and  $CT_{max}$  were similar across *Sceloporus* species (Fig. 4), the onset  $T_a$  and subsequent efficacy of panting may be dependent on the heat loads each species is exposed to in their respective habitats. Because *S. cowlesi* individuals from this study were captured at elevations ranging from 1500 to 2500 m, the high variation seen in  $T_b$  depression may be a function of local adaptation of the populations sampled (Herrando-Pérez et al., 2019). As *S. clarkii* and *S. cowlesi* are found in a wide variety of thermal habitats, this observation may reflect adherence to the climatic variability hypothesis, which states that species that persist in more variable climates will have a wider breadth of tolerances (reviewed in Gaston et al., 2009).

The maximum  $T_a$ – $T_b$  gradients sustained by *Phrynosoma* species sampled showed little variance, although like *Sceloporus* they occur in a wide range of climatic environments. For example, *P. hernandesi* were collected at elevations that ranged from 2400 m to over 3000 m, where they are not exposed to the same high  $T_a$  and environmental heat loads as their lower elevation congeners, and are more likely to maintain lower preferred  $T_b$  (Christian, 1998; Lara-Reséndiz et al., 2015). *Phrynosoma hernandesi* was, however, able to establish a maximum  $T_a$ – $T_b$  gradient of 2.3°C, comparable to that of its congeners. In comparison, at lower elevation, the desert-adapted *P. cornutum*, *P. modestum* and *P. solare* were able to establish maximum  $T_a$ – $T_b$  gradients of 2.5, 2.7 and 2.5°C,

respectively (Fig. 3A). Their similar capacities for evaporative cooling, despite their habitat differences, may be due in part to the flattened disc-like body shape of horned lizards, which tends to produce large solar heat loads. When combined with their sit-and-wait foraging habits (e.g. feeding at ant mounds), horned lizards may spend extended periods exposed to direct solar radiation and high surface temperatures (Sherbrooke, 2003).

### $M_b$ and environmental acclimatization

Although there was no significant interspecific effect of  $M_b$  in the determination of panting threshold or  $CT_{max}$ ,  $M_b$  was a significant factor in determining intraspecific panting thresholds. Table 1 illustrates how body size influenced the  $T_b$  where the onset of panting was observed. Nearly all species sampled showed a significant negative trend between body size and panting threshold, with smaller individuals generally showing elevated panting thresholds. Interestingly, while this trend was strongest in *C. collaris*, a species with a strong cooling ability, it was also observed in species that showed a limited ability to reduce  $T_b$  below  $T_a$  (e.g. *A. exsanguis*, *S. poinsettii*). Interspecifically, we could not discern a pattern that might link panting threshold to foraging mode, phylogeny or habitat. Within-species effects are likely due to the physical constraints of heat exchange, and the associated potential to lose water, as smaller individuals have higher mass-specific rates of metabolism and evaporative water loss associated with open-mouth respiration and smaller body water pools (C.L.L., unpublished data). We believe the elevated panting thresholds observed in smaller conspecifics are likely driven by the need for water conservation; such trade-offs may drive different thermoregulatory strategies, such as more frequent shuttling between thermal environments, and maintaining lower overall  $T_b$ , as seen in other squamate reptiles (Aubret and Shine, 2010; Loughran, 2014; Sears et al., 2016).

The apparent effect of environmental acclimatization on the lability of panting threshold and  $CT_{max}$  suggests the capacity for local and regional adaptation to shifting climatic conditions. However, because our lizards were captured between the spring and autumn equinoxes, the positive relationship between panting threshold and increasing acclimatization temperature may have been influenced by the extended photoperiod that lizards were exposed to as the seasons progressed, as well as acclimatization to warmer temperatures (Chong et al., 1973; Heatwole et al., 1975). Additionally, while most of our lizards were tested within the first 24 h of capture, individuals that were held for longer may have exhibited acclimation to captive conditions. Nevertheless, as the seasons progress and average heat loads increase, so does the potential for dehydration and desiccation. To avoid this, lizards may be more judicious in employing evaporative cooling during periods of prolonged heat and aridity. The significant positive relationship we observed between maximum average environmental temperature and  $CT_{max}$  is consistent with observations of short-term heat acclimation seen in amphibians exposed to prolonged heat (Hutchison and Maness, 1979), as well as intraspecific variation in  $CT_{max}$  among populations (Herrando-Pérez et al., 2019). Although the mechanism for selection is unclear, elevated environmental temperatures that result in higher  $T_e$  force lizards to either retreat to cooler microclimates or cope by extending their exposure to higher heat loads. Lizards that opt for the latter may show heat hardening (Phillips et al., 2016) and the expression of genes associated with the protection of cells. These responses include the induction of an array of heat shock proteins, the up-regulation of interleukins that affect inflammatory responses and the



downregulation of coagulation pathways (McKechnie and Wolf, 2019) and thus potentially increase their  $CT_{max}$ . Although the apparent plasticity of  $CT_{max}$  can increase thermal safety margins, factors such as latitude, taxon and  $M_b$  can interact to influence its magnitude (Rohr et al., 2018). We did not observe any of these interactive effects in our analysis, which may be due in part to the coarse nature of the climate data used. However, episodic climatic extremes, such as heat waves or drought, may be a stronger selective force for greater thermal limits than long-term exposure (Sunday et al., 2019).

### $CT_{max}$ and panting threshold

Lutterschmidt and Hutchison (1997) have advocated the use of a specific protocol to obtain consistent estimates of  $CT_{max}$ , i.e. the rapid elevation (10–30 min) of  $T_b$  to the endpoints defined by the observed LRR and/or OS (Hutchison, 1961). There is a long-running conversation on  $CT_{max}$  that has noted issues with high rates of heating, fatigue associated with flipping animals to establish LRR and the transient/spurious occurrence of OS (Licht et al., 1966). More recently, a critique of approaches for estimating thermal tolerance with recommendations has been provided by Camacho and Rusch (2017) with a focus on lizards. Their review of measurements of  $CT_{max}$  found that time of year, photoperiod, changes in mass, hydration level, housing, experimental protocols and acclimation history all produced variation in expressed  $CT_{max}$  values. We detail our methods here, because our  $CT_{max}$  values could be biased compared with those of other more conventional  $CT_{max}$  studies. Our approach differed from the established  $CT_{max}$  methodology because we wanted to measure the steady-state values for metabolism and evaporative water loss as we increased heat loads on individual animals. This approach provided us with estimates of panting threshold,  $CT_{max}$  and heat tolerance limits as well as continuous metabolic and evaporative water loss data. Our goal was to establish consistency in our trials such that we could make valid comparisons across the species we studied. To this end, we used wild-captured individuals that were engaged in trials typically within 24–48 h of capture. Because our animals were held in sealed metabolic chambers, we did not use LRR as the sole criterion for estimating  $CT_{max}$ . Rather, we used a combination of OS, rapid increases in  $T_b$ , a large drop in  $CO_2$  production and continuous escape behavior as criteria for ending a trial. The primary difference between our methodology and other recent studies (e.g. Brusch et al., 2015; Herrando-Pérez et al., 2019) is that our trials typically were 4 h in duration; most  $CT_{max}$  trials last from 10 min to 1 h. As other researchers have noted, thermal acclimation can be rapid, with significant effects on  $CT_{max}$  occurring within 1.5 h (Art and Claussen, 1982). This ‘heat hardening’ may produce increases in  $CT_{max}$  of 1–5°C and peak over periods of 1.5–30 h (Ballinger and Schrank, 1970; Art and Claussen, 1982; Phillips et al., 2016). Given these data, we believe that our 4 h exposures could have produced  $CT_{max}$  values that were ~1°C higher than those we may have obtained using standard methods (Hutchison, 1961). How do our  $CT_{max}$  values compare with those from other studies? Although comparative data are limited, we provide several examples. In *P. cornutum*, our estimated  $CT_{max}$  was  $44.8 \pm 0.1^\circ C$  (Fig. 3A, Table 1) compared with values of  $46.8^\circ C$  (Kour and Hutchison, 1970),  $47.9^\circ C$  (Prieto and Whitford, 1971) and  $45.1^\circ C$  after 30 h of conditioning at  $T_a = 27^\circ C$ , and  $45.9^\circ C$  after 30 h of conditioning at  $T_a = 40^\circ C$  (Ballinger and Schrank, 1970). Our  $CT_{max}$  estimate of  $47.4^\circ C$  for *D. dorsalis* closely matches the value of  $47.5^\circ C$  obtained by Cowles and Bogert (1944) and our  $CT_{max}$  estimate of  $45.4^\circ C$  for *P. hernandesi* is very close to that of  $45.5^\circ C$

obtained by Prieto and Whitford (1971). A study by Smith and Ballinger (1994) of desert and low montane populations of *U. ornatus* in southern New Mexico and southeastern Arizona found a common  $CT_{max}$  value among populations that varied from month to month and ranged from  $38.4$  to  $46.2^\circ C$  with a mean value of  $43.3^\circ C$ . Our values for *U. ornatus* averaged  $45^\circ C$ . While the literature data preclude species by species comparisons with our data, we believe that these examples illustrate the comparability of our approaches with classic methodologies for estimating  $CT_{max}$ . We still urge caution in making definitive comparisons of  $CT_{max}$  across datasets where any number of environmental, biological or experimental variables may introduce bias (Camacho and Rusch, 2017).

One of our goals was to examine whether panting threshold could serve as a robust predictor of  $CT_{max}$  within species and examine the variation across species. Recent studies have highlighted the importance of measuring other thermal traits, such as  $T_{pref}$  and voluntary thermal maximum in relation to  $CT_{max}$ , as they provide important context to the level of thermal stress animals may be experiencing (Camacho and Rusch, 2017; Camacho et al., 2018). If panting threshold is a robust predictor of  $CT_{max}$  then it could provide a sublethal metric of extreme thermal stress. We found significant variation in panting threshold– $CT_{max}$  values among species with panting threshold– $CT_{max}$  differentials that ranged from  $1.9^\circ C$  in *A. exsanguis* to  $5^\circ C$  in *P. hernandesi* (Table 1). Within genera, we found a fairly consistent differential between panting threshold and  $CT_{max}$  with values for five *Sceloporus* species, for example, averaging  $4.2^\circ C$  and ranging from  $3.7$  to  $4.7^\circ C$  (Fig. 4A). In four *Phrynosoma* species, panting threshold– $CT_{max}$  values averaged  $4.6^\circ C$  and ranged from  $4.0$  to  $5.2^\circ C$ . The range of variation we observed for panting threshold– $CT_{max}$  among individuals within a species was  $3.5$ – $5.5^\circ C$ . Most of the observed variance in the panting threshold– $CT_{max}$  differential is associated with variation in panting threshold. The observed range of variation in panting threshold within a species suggests caution should be used in applying panting threshold as a direct proxy for  $CT_{max}$ . The within-species variation we observed is consistent with that reported in Heatwole et al.’s (1973) review of the literature on panting and gaping thresholds in reptiles where within-species range for the onset of panting averaged  $3.9^\circ C$  and varied from  $0.5$  to  $10.7^\circ C$  across 33 lizard species (Table 1). A number of researchers have elaborated on the sources of this variation; Heatwole et al. (1973) found that body size, sex and the method of heating had no effect on the measured panting threshold in *Amphibolurus muricatus* (now *Pogona muricatus*). However, rapid heating and non-radiant heat sources increased the variation in the measured panting threshold and the primary source of variation in panting threshold was day-to-day shifts within individuals. Parmenter and Heatwole (1975) extended this work looking at hydration status and its effects on panting threshold in *Amphibolurus barbatus* (now *Pogona barbatus*) and *A. muricatus*. They found that *A. barbatus* elevated its panting threshold by as much as  $4^\circ C$  with increasing dehydration, which was not observed in *A. muricatus*. Increases in panting threshold with dehydration in *A. barbatus* were seen as a water conservation strategy because of its occupancy of more arid habitats than *A. muricatus*. da Silveria Scarpellini et al. (2015) used salt loading as a proxy for dehydration stress to examine the mechanisms underlying thermoregulation in *Pogona vitticeps*. They found that progressive increases in plasma osmolality greatly reduced the gaping response, indicating that water conservation is likely a strong driver of thermoregulatory behavior. Other environmental sources of variation affecting panting threshold



include both diel and seasonal effects as reported by Chong et al. (1973) and Heatwole et al. (1975). Given the dynamic nature of these variables, especially among species, it is unsurprising that we did not observe a significant relationship between panting threshold and  $CT_{max}$ . However, we believe that panting threshold can provide an important, measurable benchmark indicating extraordinary thermal stress and provide important context for both field and laboratory studies (Dewitt, 1967; Tattersall et al., 2006).

### Thermal limits and phylogeny

Panting threshold and  $CT_{max}$  values showed significant phylogenetic signal, indicating these traits are conserved within their respective clades, therefore suggesting that selection for higher upper thermal limits has progressed more slowly in certain clades. Although we lack data on voluntary thermal maxima for many of these species, this result may align with the suggestions of other studies that the Bogert effect (the behavioral avoidance of higher temperatures that results in decreased selection of upper thermal limits) hinders the evolution of the upper thermal tolerances required for lizard species to persist amidst climate warming (Huey et al., 2003; Buckley et al., 2015; Domínguez-Guerrero et al., 2019; Muñoz and Bodensteiner, 2019). Interestingly, the maximum  $T_a - T_b$  gradient maintained while panting did not show a significant phylogenetic signal (Fig. 3B), which may indicate that adaptive thermoregulatory pressures may be lower on species that infrequently experience higher environmental heat loads, such as those in high elevations. Wiens et al. (2019), for example, observed that the lower  $T_{pref}$  of *S. jarrovi* populations and their occupancy of cool habitats in Arizona's sky islands likely left them more isolated than the more thermophilic sympatric *S. clarkii* when climates have warmed. The absence of *S. jarrovi* in the lower, more arid environments may have resulted from their behavioral avoidance of high heat loads, thereby alleviating selective pressure to effectively evaporatively cool. In contrast, *S. clarkii*, a species that has a higher thermal tolerance and can efficiently evaporatively cool, is better equipped to tolerate high ambient heat loads, and therefore can exploit a wider range of thermal habitats.

### Thermoregulation and climate change

We have provided data showing the extent to which panting can lower  $T_b$  for a variety of arid-adapted lizard species. This has important implications for making predictions about lizard extinction risk, as models that incorporate lizard thermoregulation are built around the premise that ectotherm activity and thus energy budgets are constrained by thermal thresholds. While these models generally predict dire consequences, they do not typically account for panting as a thermoregulatory mechanism, nor its potential to extend activity periods via evaporative cooling (Sinervo et al., 2010; Huey et al., 2012; Gunderson and Leal, 2015). Recent studies have suggested that thermal refugia on the landscape will become more clumped, increasing intraspecific competition for ideal microclimates, and further adding to the hormonal and energetic costs associated with effective regulation of a  $T_{pref}$  (Sears et al., 2016; Basson et al., 2017; Rusch and Angilletta, 2017; Rusch et al., 2018). However, these models account for only the 'thermal shuttling' perspective, and do not include physiological parameters such as evaporative cooling potential, shifts in thermal optima (i.e. 'acclimatization') or ontogenetic shifts in energy and water usage. Because panting is inherently dependent on the endogenous water reserves a lizard has, availability of water on the landscape (as either food or free-standing water) will certainly constrain the use of panting as a thermoregulatory mechanism. Although water reserves

are not typically accounted for, recently there has been increasing awareness of the need to incorporate water budgets, and the associated potential for evaporative cooling or desiccation, into activity budgets and extinction risk predictions (Sunday et al., 2014; Riddell et al., 2017; Kearney et al., 2018; Rozen-Rechels et al., 2019; Huang et al., 2020).

Given that lizard activity is constrained when thermal conditions are unsuitable, panting has the potential to extend activity periods and mitigate this effect (provided there are sufficient water reserves). Species that can significantly lower their  $T_b$  below  $T_a$  are well equipped to avoid lethal environmental extremes, tolerate prolonged heat exposure, and possibly exploit thermal environments unavailable to competitors. One such case discussed above is the stark difference between the heat-tolerant *S. clarkii* and its congener *S. jarrovi*; the apparent ability of *S. clarkii* to use panting to lower its  $T_b$  may give it a competitive edge in thermal transition zones where it overlaps with other *Sceloporus* (Wiens et al., 2019). The expanded activity time, especially in differing habitats, allows for greater energy consumption and assimilation (Karasov and Anderson, 1984). Similarly, other species with high evaporative cooling capacities (e.g. *Phrynosoma*, *Crotaphytus*) may exploit habitats that become available as thermal niches shift, potentially shifting segments of their geographic or elevational range (Sinervo et al., 2010; Chen et al., 2011).

For dry-skinned ectotherms, such as lizards, it is crucial to understand how evaporative cooling is used and whether it is indeed a practical strategy for a given species. We have shown here that for some species it may be a useful mechanism for dissipating environmental heat loads, while it is likely to be ineffective for other species. We submit that in addition to current existing models, physiological, ecological and behavioral aspects must be considered for each species when assessing its risk of extinction (Urban et al., 2016). Employing mechanistic models, which favor individual-based eco-physiological parameters, should provide more robust predictive power when projecting lizard extinction risk (Kearney and Porter, 2009; Angilletta et al., 2019).

### Acknowledgements

We thank Chris Anderson, Lauren Bansbach, Bruce Christman and J. Tom Giermakowski for invaluable assistance in locating and capturing lizards in the field, and Chris R. Tracy for providing the chuckwallas. Chris Anderson was also helpful in phylogenetic analyses. We also thank two anonymous reviewers, whose comments helped to significantly improve the manuscript.

### Competing interests

The authors declare no competing or financial interests.

### Author contributions

Conceptualization: C.L.L., B.O.W.; Methodology: C.L.L., B.O.W.; Formal analysis: C.L.L.; Investigation: C.L.L.; Resources: B.O.W.; Data curation: C.L.L.; Writing - original draft: C.L.L.; Writing - review & editing: C.L.L., B.O.W.; Visualization: C.L.L.; Supervision: B.O.W.; Funding acquisition: C.L.L., B.O.W.

### Funding

Funding to C.L.L. was provided by the University of New Mexico BGSA Research Allocations Committee, the University of New Mexico GPSA Student Research Grant and New Mexico Research Grant, the Melinda Bealmer Memorial Scholarship, and the Alvin R. and Caroline G. Grove Summer Research Scholarship. This work was also supported by National Science Foundation grant DEB 1457524 to B.O.W. Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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