

RESEARCH PAPER

Body size impacts critical thermal maximum measurements in lizards

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Abstract

Understanding the mechanisms behind critical thermal maxima (CT_{max}; the high body temperature at which neuromuscular coordination is lost) of organisms is central to understanding ectotherm thermal tolerance. Body size is an often overlooked variable that may affect interpretation of CT_{max}, and consequently, how CT_{max} is used to evaluate mechanistic hypotheses of thermal tolerance. We tested the hypothesis that body size affects CT_{max} and its interpretation in two experimental contexts. First, in four *Sceloporus* species, we examined how inter- and intraspecific variation in body size affected CT_{max} at normoxic and experimentally induced hypoxic conditions, and cloacal heating rate under normoxic conditions. Negative relationships between body size and CT_{max} were exaggerated in larger species, and hypoxia-related reductions in CT_{max} were unaffected by body size. Smaller individuals had faster cloacal heating rates and higher CT_{max}, and variation in cloacal heating rate affected CT_{max} in the largest species. Second, we examined how body size interacted with the location of body temperature measurements (i.e., cloaca vs. brain) in *Sceloporus occidentalis*, then compared this in living and deceased lizards. Brain temperatures were consistently lower than cloacal temperatures. Smaller lizards had larger brain-cloacal temperature differences than larger lizards, due to a slower cloacal heating rate in large lizards. Both live and dead lizards had lower brain than cloacal temperatures, suggesting living lizards do not actively maintain lower brain temperatures when they cannot pant. Thermal inertia influences CT_{max} data in complex ways, and body size should therefore be considered in studies involving CT_{max} data on species with variable sizes.

KEYWORDS

body size, critical thermal maximum, hypoxia, thermal tolerance

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1 | INTRODUCTION

As climate change progresses rapidly, there is an increased urgency in advancing understanding of the impacts of body temperature on physiological and behavioral processes in ectotherms (Brusch, Taylor, & Whitfield, 2016; Clusella-Trullas, Blackburn, & Chown, 2011; Parmesan, 2006; Sinervo et al., 2010; Thomas et al., 2004). Global temperatures are projected to rise 0.8°C–2°C by 2050 (Collins et al., 2013), and these projected increases in environmental temperatures are predicted to alter thermoregulation and habitat selection of ectotherms (reviewed in Angilletta, Niewiarowski, & Navas, 2002; reviewed in Huey & Stevenson, 1979). Additionally, ectotherms may respond by changing their range by increases in elevation or latitude (Diele-Viegas & Rocha, 2018; Sunday, Bates, & Dulvy, 2012). While behavioral plasticity and range expansion may provide a thermal buffer, environmental temperature extremes can directly and significantly influence body temperatures, affecting biochemical and physiological processes important for growth, reproduction, and locomotion (Cloudsley-Thompson, 1999; Dawson, 1975). Due to enhanced risk of ectotherm survival at extreme environmental temperatures, there is renewed interest in quantifying the upper thermal tolerance of ectotherm species and examining the physiological mechanisms behind these animals' responses to high temperatures.

A useful measure of upper thermal tolerance is the critical thermal maxima (CT_{max}), the upper temperature at which an organism is able to maintain physiological functions before reaching its upper lethal temperature (Cowles & Bogert, 1944). While CT_{max} has recent applications in projecting species distribution and survival in the context of global warming (Kearney & Porter, 2009; reviewed by Taylor et al., 2020), CT_{max} has been featured extensively in the study of mechanistic hypotheses describing upper thermal limits (e.g., Frederick & Pörtner, 2000; Gangloff & Telemeco, 2018; Paulson & Hutchinson, 1987). One hypothesis posits that CT_{max} is reached when an organism is unable to deliver a sufficient amount of oxygen to body tissues as physiological processes accelerate with rising temperatures (the oxygen- and capacity-limited thermal tolerance hypothesis or OCLTT; Frederick & Pörtner, 2000). Alternatively, CT_{max} may be caused by the impact of high temperatures on muscle and nervous tissue, impairing whole-body function (Paulson & Hutchinson, 1987). Another hypothesis effectively marries the two ideas by stating that oxygen limitation only determines CT_{max} at extreme hypoxia, while temperature sensitivity of enzymes and other proteins may be more important under normoxic conditions (Hierarchical mechanisms of thermal limitation hypothesis or HMTL; Gangloff & Telemeco, 2018). A multitude of studies have also examined other factors that impact CT_{max}, ranging from diel and seasonal variation to acclimation to laboratory conditions (reviewed in Lutterschmidt & Hutchison, 1997), yet the underlying physiological mechanisms of CT_{max} are still not fully resolved.

Lizards have proven to be rich model organisms for studies of thermal tolerance (Camacho & Rusch, 2017). The CT_{max} is easily observed, marked by a loss of righting response that is sometimes

followed by an onset of muscular spasms (Lutterschmidt & Hutchison, 1997); CT_{max} is also easily measured by inserting a thermal probe into the cloaca. In lizards, there is general support for OCLTT and/or HMTL hypotheses; numerous studies have demonstrated that exposure to hypoxic air causes lizards to select lower body temperatures and exhibit gaping and panting (to reduce body temperature via evaporative cooling) at lower temperatures than when breathing normoxic gas (Branco, Gargaglioni, & Barros, 2006; DuBois, Shea, Claunch, & Taylor, 2017; Petersen, Gleeson, & Scholnick, 2003; Shea et al., 2016). Additionally, adult lizards treated with severely hypoxic gas showed reduced CT_{max} (DuBois et al., 2017; Shea et al., 2016), and lizard embryos treated with hypoxic gas exhibited reduced survival (Smith, Telemeco, Angilletta, & VandenBrooks, 2015), but oxygen does not appear to limit thermal tolerance under mildly hypoxic or normoxic conditions (Camacho, Vandenbrooks, Riley, Telemeco, & Angilletta, 2018). There is less empirical support for upper thermal limit hypotheses under normoxic conditions, and much remains to be tested before CT_{max} is well-understood in lizards.

Regardless of the mechanism of CT_{max} itself, it is prudent to understand how upper thermal tolerance measurements are affected by experimental context and procedures. Recent multispecies database studies have found that CT_{max} may vary with latitude, elevation, and body size (Leiva, Calosi, & Verberk, 2019; Rubalcaba & Olalla-Tárraga, 2020; Sunday et al., 2019). However, the phylogeny of species in the analysis often predicts thermal limits better than geography or body size (Leiva et al., 2019; Rubalcaba & Olalla-Tárraga, 2020). This is likely due to the nature of these studies, namely using a single averaged value for size, distribution, and thermal limits for each species in the analyses. Even at this coarse scale, these studies emphasize the importance of context-dependence in support for different mechanistic hypotheses. To use the CT_{max} in future studies, it is important to consider covariates that influence interpretations of CT_{max} within and among species, such as body size (Rubalcaba & Olalla-Tárraga, 2020). Shea et al. (2016) demonstrated a strong impact of body size under extreme hypoxia in *Sceloporus occidentalis* (Baird & Girard, 1852), where lizard snout-vent length (SVL) was significantly and negatively related to CT_{max}. The mechanisms responsible for size-dependent effects on CT_{max} in lizards are unknown, but may relate to a number of factors. If oxygen limitation is important in determining CT_{max}, greater energy expenditure coupled with potential limitations in oxygen transport in larger lizards (Gillooly, Gomez, Mavrodiev, Rong, & McLaure, 2016; Roe, Hopkins, & Talent, 2005; Zari, 1991) may result in a lower CT_{max}. Heating rate, known to influence CT_{max} (Sunday et al., 2019), may be impacted by thermal inertia resulting from large body sizes. Additionally, thermal inertia may affect thermal insulation at locations where body temperatures are measured (Camacho & Rusch, 2017) in different sized lizards, even when ambient temperature heating rate remains constant. If the most common location of lizard body temperature measurements—the cloaca—is more insulated or more affected by thermal inertia in larger lizards than temperature of a more relevant tissue, such as the brain, then observations of different CT_{max} based on cloacal

temperatures among animals of different sizes may be artifacts that plague interpretation of the CT_{max}.

We hypothesized that body size affects upper thermal tolerance measurements and its corresponding interpretation in lizards. We tested this main hypothesis in two experimental contexts. Our first experiment examined CT_{max} in multiple species of lizards that vary in body size in the context of the OCLTT hypothesis. We tested CT_{max} in four species of *Sceloporus* lizards occurring at varying elevations at normoxia (i.e., the naturally occurring partial pressure of oxygen at each elevation) and experimentally induced extreme hypoxia. In accordance with OCLTT, we predicted that larger species and larger individuals would have lower CT_{max}, owing to reduced oxygen transport capacity in larger individuals due to lower relative surface area for oxygen uptake and transport to tissues (Gillooly et al., 2016). Additionally, in accordance with HMTL we predicted that at lower partial pressures of oxygen (i.e., at higher elevations and under extreme hypoxia), all lizards would have lower CT_{max}, and that hypoxia would have more extreme negative effects on CT_{max} of larger lizards. Finally, we explored how lizard heating rate and body mass interacted within and among species to affect CT_{max} under normoxic conditions. We predicted that due to thermal inertia, the cloaca of large lizards would heat more slowly, resulting in lower observed CT_{max}. Our second experiment examined the influence of the location of body temperature measurements (e.g., cloaca vs. brain) and the corresponding influence of physiological thermal buffering on heating rates within *S. occidentalis*. To test this, we assessed responses to ambient heating *via* temperature of thermal probes in the cloaca and brain of lizards of varying body sizes when they were alive (to include any effects of physiological thermoregulation due to vasomotor or other changes), then repeated the procedure in the same lizards after they were deceased (to include only inertial effects). If lizards show physiological thermoregulation of brain temperature, we predicted that brain temperatures would be lower than cloacal temperatures in all lizards when alive but not when dead. Due to effects of thermal inertia, we predicted there would be a larger difference between brain and body measurements in larger individuals in both living and dead lizards. These data are critical in understanding how variation in body size can impact the CT_{max} data that researchers often collect (Camacho & Rusch, 2017) and the wider interpretation of CT_{max} data when applied in conservation contexts (Kearney & Porter, 2009).

2 | MATERIALS AND METHODS

2.1 | Experiment 1: Effects of body size and oxygen on CT_{max} and cloacal heating rates in four *Sceloporus* species

2.1.1 | Study sites and species

We collected data from four lizard species at nine field sites in California and Arizona, during the spring and summer months of

2015–2016 (Table 1). We studied these species because they are abundant, easily captured, heliothermic lizards that vary dramatically in size (SVL), with *Sceloporus jarrovi* (Cope, in Yarrow, 1875) the largest, followed by *S. occidentalis*, *Sceloporus virgatus* (Smith, 1938), and *Sceloporus graciosus* (Baird & Girard, 1852). Lizards were collected by hand or lasso and placed into individual numbered tube socks until measurement. Mass and SVL were recorded. CT_{max} measurements were conducted the day of capture to minimize acclimation effects (e.g., Yang, Sun, An, & Ji, 2008; see below for details), and lizards were released at their sites of capture the same or next day. Data from lizards collected in 2015 are previously reported in Shea et al. (2016) and DuBois et al. (2017). All procedures were approved by the Institutional Animal Care and Use Committee of the California Polytechnic State University (protocol #1506), the California Department of Fish and Wildlife (CDFW Scientific Collecting Permit #13265), and the Arizona Game and Fish Department (AZGF Scientific Collecting Permit #753254).

2.1.2 | Procedures

To measure CT_{max} of the lizards, we used the field-portable Controlled Reptile Oxygen and Climate System (CROCS; see Shea et al., 2016). Briefly, CROCS convectively heats six lizards inside individual test chambers via an Arduino Uno R3 microcontroller with custom gas mixtures facilitating study of responses to hypoxic gas. Lizard cloacae were fitted with resistance temperature detectors and placed in individual chambers. Detectors were coated with a water-proof and highly thermally conductive epoxy that measures body temperature as chambers are heated. Before beginning the experiment, all chambers were heated until lizard body temperatures matched the chamber temperature of 30°C. Then, lizards were exposed to either normoxic gas (21% oxygen, 79% nitrogen) from a compressor or hypoxic gas (6% oxygen, 94% nitrogen) from a scuba tank, which was delivered at a constant ambient heating rate of 1°C/min, the most common ramping rate for thermal limit studies (as compiled in Sunday et al., 2019). The nine field sites range from 0 to 2,680 m in elevation, so the atmospheric pressure and therefore partial pressures of oxygen in each treatment vary among sites (Table 1). Once lizards began panting, we rapidly rotated each individual chamber such that lizards were flipped onto their backs and then righted themselves. We flipped lizards in this manner approximately five times/min until they lost their righting response; this temperature represented CT_{max}. Lizards were immediately removed from the chambers and cooled.

2.1.3 | Data analysis

Data were checked for assumptions of normality, homoskedasticity, and homogeneity of variances. Mass was log-transformed to improve model fit. To assess the impacts of body mass within and among species of differing body size ranges under varying normoxic and

TABLE 1 Descriptive data on study species and characteristics of study sites for Experiment 1

Species	Study Site	Elevation (m)	Date(s)	Treatment and P _{O2} (mmHg)	N	Mean mass (g) ±1 SEM	Mean CT _{max} (°C) ±1 SEM
<i>Sceloporus graciosus</i>	Poison Creek Campground, California	2,100	July 2015–2016	Hypoxic (6% O ₂) (P _{O2} = 35.8)	11	6.8 ± 0.4	37.5 ± 0.7
				Normoxic (21% O ₂) (P _{O2} = 125.4)	34	7.1 ± 0.2	42.6 ± 0.4
<i>S. graciosus</i>	Round Meadow, California	2,680	July 2015–2016	Hypoxic (6% O ₂) (P _{O2} = 33.5)	11	7.0 ± 0.4	34.7 ± 0.5
<i>Sceloporus jarrovi</i>	Portal, Arizona	1,645	August 2016	Normoxic (21% O ₂) (P _{O2} = 117.2)	29	6.9 ± 0.2	42.3 ± 0.3
				Hypoxic (6.4% O ₂) (P _{O2} = 40.0)	26	13.4 ± 1.1	30.7 ± 0.4
<i>Sceloporus occidentalis</i>	Cal Poly campus, San Luis Obispo, California	0	July 2015–2016	Normoxic (21% O ₂) (P _{O2} = 132.3)	26	11.1 ± 0.7	35.4 ± 0.7
				Hypoxic (6% O ₂) (P _{O2} = 45.6)	25	11.1 ± 0.4	35.0 ± 0.5
<i>S. occidentalis</i>	Hospital Flats Campground, California	900	July 2015	Normoxic (21% O ₂) (P _{O2} = 159.6)	69	11.8 ± 0.3	41.4 ± 0.3
				Hypoxic (6% O ₂) (P _{O2} = 41.2)	10	15.7 ± 1.1	39.3 ± 0.4
<i>S. occidentalis</i>	Kern River Trailhead, California	1,475	July 2015	Normoxic (21% O ₂) (P _{O2} = 144.0)	12	15.0 ± 1.2	42.2 ± 0.4
				Hypoxic (6% O ₂) (P _{O2} = 38.6)	6	12.5 ± 1.6	36.8 ± 0.7
<i>S. occidentalis</i>	Poison Creek Campground, California	2,100	July 2015	Normoxic (21% O ₂) (P _{O2} = 135.0)	12	11.5 ± 1.0	42.6 ± 0.9
				Hypoxic (6% O ₂) (P _{O2} = 35.8)	6	12.6 ± 2.3	33.9 ± 0.8
<i>Sceloporus virgatus</i>	Portal, Arizona	1,645	August 2016	Normoxic (21% O ₂) (P _{O2} = 125.4)	5	11.9 ± 2.2	42.8 ± 0.3
				Hypoxic (6.4% O ₂) (P _{O2} = 40.0)	14	6.5 ± 0.3	33.2 ± 0.8
<i>Sceloporus virgatus</i>	Portal, Arizona	1,645	August 2016	Normoxic (21% O ₂) (P _{O2} = 132.3)	11	5.8 ± 0.4	38.2 ± 0.9
				Hypoxic (6.4% O ₂) (P _{O2} = 40.0)	14	6.5 ± 0.3	33.2 ± 0.8

Note: Species sampled in 2015 are discussed in detail in Shea et al. (2016) and DuBois et al. (2017).

hypoxic conditions, we ran a linear mixed effects model using the lme function in the “nlme” package in R (Pinheiro, Bates, DebRoy, Sarkar, & Core Team, 2017; v3.1.131; R Core Team, 2017; v 3.4.0 Patched). The following interactions were included to assess their influence on the response CT_{max}: species and mass, species and experimental P_{O₂}, mass and experimental P_{O₂}. Main effects of species, mass, and experimental P_{O₂} were also included in the model. Experimental P_{O₂} refers to the P_{O₂} experienced by the lizards during testing, with higher values representing normoxia and lower values representing experimentally induced hypoxia (outlined in Table 1). To account for site-specific differences that may have affected any of our measured variables, site was included in the analysis as a random effect. Numbers of individuals for each species, treatment, and site are reported in Table 1. A subset of data (normoxia only, which represents the actual oxygen concentrations lizards experience at each site) were used to investigate the hypothesis that mass and cloacal heating rate interact to affect CT_{max} (*N* = 63 *S. graciosus*, *N* = 26 *S. jarrovi*, *N* = 97 *S. occidentalis*, *N* = 11 *S. virgatus*). Cloacal heating rate was calculated as the change in cloacal temperature from the experiment's start to the CT_{max}, divided by the total time (in minutes) to reach CT_{max}. The following interactions were included to assess their influence on the response CT_{max}: species and mass (to assess interspecies effects of body size at normoxia), species and cloacal heating rate (to assess inter-species effects of cloacal heating rate at normoxia), and mass and cloacal heating rate (to assess inter-individual effects of body size and body heating rate on CT_{max} at normoxia). Main effects of species, mass, and cloacal heating rate were included in the model. Site was included as a random effect for reasons described above. For both the full and normoxia-subsetted data, we conducted AIC model selection on sets of models including those described above and simpler iterations of those models. The models described above returned the lowest AIC value or were indistinguishable from any simpler iterations (within Δ2 of the lowest AIC).

2.2 | Experiment 2: Brain-cloaca temperature differential in *S. occidentalis*

2.2.1 | Study sites and species

These experiments were conducted on *S. occidentalis* from the campus of the California Polytechnic State University in San Luis Obispo, California (*N* = 14) and the Chimineas Ranch in the Carrizo Plain (*N* = 2). Lizards were captured and measured as above and tested the same day as capture.

2.2.2 | Procedures

Each lizard was anesthetized with a cotton ball soaked in isoflurane (VetOne® Fluriso™ 99.9%), pressed to the snout, and secured in place via a cut-off glove fingertip. A lizard was considered thoroughly

anesthetized when pinching their leg failed to elicit a withdrawal response. Once anesthetized, a thermocouple was inserted into the cloaca and secured in place by medical tape wrapped around the base of the tail. To measure brain temperature, a 21g hypodermic needle was used to pierce the skin and skull to give access to the brain. Once pierced, a thermocouple was inserted into the brain and secured in place with tape. Insertion location was earlier validated on a dead lizard and was consistent among all lizards. Each lizard was then placed into individual chambers in the Gas and Temperature Oxygen Regulation System (GATORS), which is a second-generation version of CROCS that uses convective heat to alter the temperature of five chambers simultaneously at a constant rate. In this experiment, cloacal and brain temperature data were continuously collected as lizards were heated from 30°C to a cloacal temperature past their upper lethal temperature (>50°C), which effected euthanasia. Chamber heating rate was a constant 1°C/min. Next, nine of the lizards (now dead and cooled to room temperature) were randomly selected to be re-ran on GATORS. Cotton balls were soaked in water to mimic the isoflurane in the previous experiment, and the glove “hood” was secured back in place with tape. The procedure was repeated as above. All procedures were approved by the Institutional Animal Care and Use Committee of the California Polytechnic State University (protocol #1707) and the California Department of Fish and Wildlife (CDFW Scientific Collecting Permit #13265).

2.2.3 | Data analysis

Data were checked for assumptions of normality, homoskedasticity, and homogeneity of variances. To investigate differences in thermal responses of brain versus body to heating in living lizards, a linear mixed effects model was run using the lme function in R (Pinheiro et al., 2017; v 3.1.131; R Core Team, 2017; v 3.4.0 Patched). The response variable body temperature included all lizard temperature measurements (both brain and cloaca). The following interactions were included to assess their influence on the response body temperature: temperature location and mass, temperature location and tube temperature, and tube temperature and mass. Main effects of temperature location, mass, and tube temperature were included in the model. Lizard ID was included as a random effect to account for repeated sampling of individual lizards (*N* = 17 lizards with 21 thermal recordings from both brain and cloaca; Figure 4). To evaluate potential differences in physiological control of temperature in brain and cloaca, we investigated brain versus body temperature differences in living and dead lizards (=status). The following interactions were included to assess their influence on the response body temperature (described above): status and temperature location, status and mass, temperature location and mass, and mass and tube temperature. Main effects of temperature location, status, mass, and tube temperature were included in the model. Lizard ID was included as a random effect to account for repeated sampling of individual lizards (*N* = 9 lizards with 19–21 paired thermal recordings from both brain and cloaca when both living and dead; Figure 5). For both the

living-only and living and dead data, we conducted AIC model selection on sets of models including those described above and simpler iterations of those models. The models described above returned the lowest AIC value or were indistinguishable from any simpler iterations (within $\Delta 2$ of the lowest AIC).

3 | RESULTS

3.1 | Experiment 1: Body size and oxygen effects on CTmax and cloacal heating rates

There was an interaction between species and mass ($F_{3,287} = 2.8$, $p = .04$), where *S. jarrovi* had the most extreme negative relationship between mass and CTmax among the species (Figure 1). Overall, with data from normoxic and hypoxic conditions combined, there was a negative relationship between mass and CTmax ($F_{1,287} = 31.0$, $p < .001$). We did not find evidence for partial pressure of oxygen influencing the effect of mass on CTmax ($F_{1,287} = 1.0$, $p = .33$), nor did we find evidence of species influencing the effect of partial pressure of oxygen on CTmax ($F_{3,287} = 2.1$, $p = .10$). CTmax increased as partial pressure of oxygen increased for all species ($F_{1,287} = 358.1$, $p < .001$; Figure 2). The main effect of species influenced CTmax, regardless of mass or partial pressure of oxygen ($F_{3,287} = 18.4$, $p < .001$). Tukey's posthoc tests revealed *S. jarrovi* had a lower CTmax on average than all other species, across both normoxic and hypoxic conditions (all $p < .05$, Table 1).

In the subsetted normoxia data, there was a significant species by mass interaction on CTmax ($F_{3,177} = 3.86$, $p = .01$), such that *S.*

virgatus had a positive slope (Figure 1a, larger masses attaining higher CTmax), significantly different from the other species' negative slopes (smaller masses attaining higher CTmax). We also observed an interaction effect of cloacal heating rate and species on CTmax ($F_{3,177} = 5.10$, $p = .002$), with the steepest positive slope observed in *S. jarrovi*; this slope was significantly different from *S. occidentalis* (Figure 3). In general, cloacal heating rate interacted with mass to affect CTmax ($F_{1,177} = 5.47$, $p = .02$), with lizards with lower mass having faster cloacal heating rates. Overall, faster heating rates independently led to higher CTmax ($F_{1,177} = 250.4$, $p < .001$), and lower masses were marginally related to higher CTmax ($F_{1,177} = 3.0$, $p = .08$). As in the previous full data set, the main effect of species influenced CTmax ($F_{3,177} = 56.1$, $p < .001$), with *S. jarrovi* having a lower CTmax than all other species (all $p < .01$).

3.2 | Experiment 2: Brain-cloacal temperature differential

While alive, cloacal temperatures were higher than brain temperatures at all ambient (tube) temperatures (Figure 4; $F_{1,692} = 296.6$, $p < .001$). Both cloacal and brain temperatures increased as ambient temperature increased ($F_{1,692} = 14,181.2$, $p < .001$), and we found no evidence that these variables behaved differently as ambient (tube) temperature increased ($F_{1,692} = 1.1$, $p = .30$). Location of temperature measurement (brain vs. cloaca) interacted with mass ($F_{1,692} = 160.8$, $p < .001$), with smaller lizards having higher temperatures overall, and the relationship between mass and cloacal temperature was more pronounced than mass and brain temperature (slopes of -0.25 vs. -0.08 , respectively, $p < .001$). There was also an interaction between tube temperature and mass, such that smaller lizards heated faster than larger lizards ($F_{1,692} = 14.4$, $p = .0002$).

All body temperatures (i.e., brain and cloaca temperatures considered together) were slightly higher when lizards were dead than when they were alive (Figure 5; $F_{1,724} = 12.757$, $p = .0004$). There was an interaction between status (live vs. dead) and mass ($F_{1,724} = 37.8$, $p < .001$) on lizard body temperatures; live lizards had a stronger negative relationship between size and body temperatures than dead lizards (slope -0.23 vs. -0.15 , respectively, $p < .001$). However, we did not observe an interaction between status and location of temperature measurement on body temperature ($F_{1,724} = 3.3$, $p = .07$), meaning that active regulation of brain or cloacal temperatures in live lizards was minimal. There was an interaction between temperature location and mass ($F_{1,724} = 101.9$, $p < .001$); cloacal temperature had a more negative relationship with size than did brain temperature (slope -0.25 vs. -0.13 , respectively, $p < .001$).

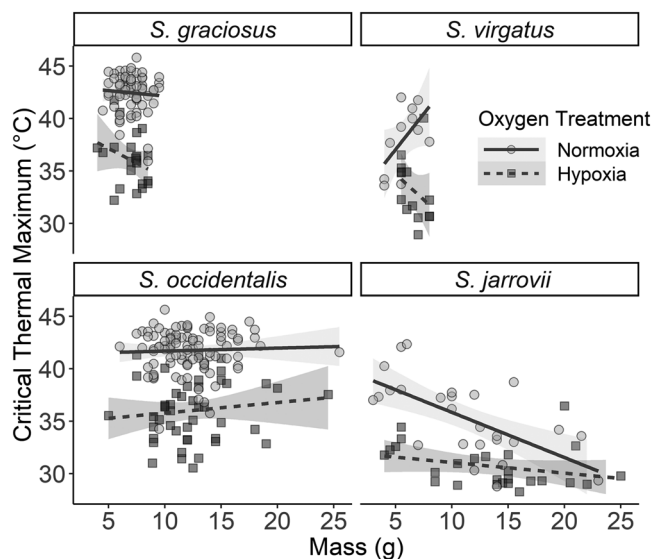


FIGURE 1 The effect of body mass on the critical thermal maximum in four species of *Sceloporus* lizards, under normoxic (circles) and severely hypoxic (squares) conditions. Sympatric species are aligned in each column. There was a negative relationship between mass and CTmax and an interaction between species and mass (with *Sceloporus jarrovi* CTmax most impacted by mass). Shaded area is 95% confidence interval

4 | DISCUSSION

Generally, we observed inverse relationships between body size and CTmax within different *Sceloporus* species in both experiments. Of note, we observed some species-specific effects on CTmax,

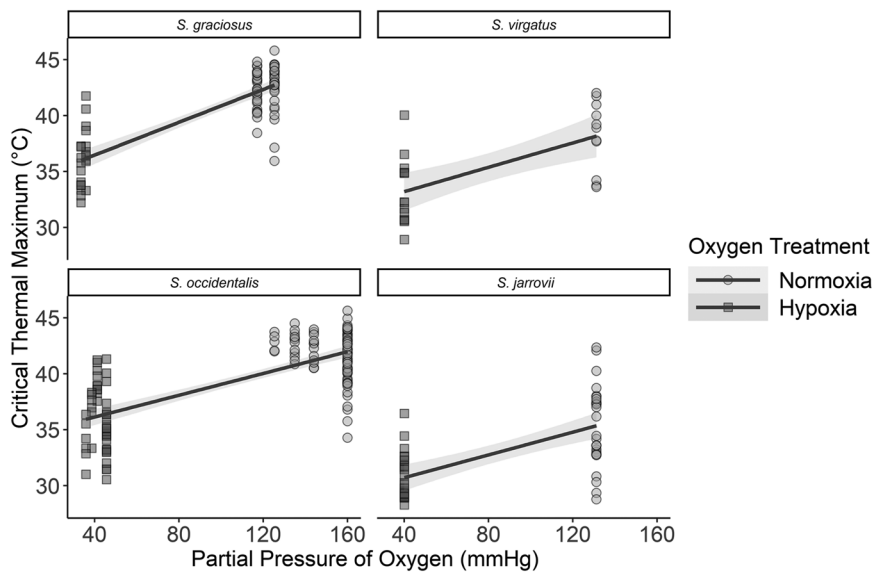


FIGURE 2 The effect of oxygen partial pressure on the critical thermal maximum in four species of *Sceloporus* lizards under normoxic (circles) and experimentally induced hypoxic (squares) conditions. CTmax increased with partial pressure of oxygen for all species, and the main effect of species influenced CTmax, regardless of mass or partial pressure of oxygen. Sympatric species are aligned in each column some of the field sites (see Table 1 for details). Shaded area is 95% confidence interval

regardless of mass or partial pressure of oxygen, which we will address before discussing within-species generalities. Rather than body size, among-species differences in CTmax could be explained by several factors. The species reaching the largest body sizes, *S. jarrovi*, had the lowest CTmax, as we predicted. However, this lower CTmax may reflect *S. jarrovi*'s activity during cold winter months when it often comes out from crevices to thermoregulate in solar radiation during the winter (Ruby, 1977; Tinkle & Hadley, 1977), unlike the other three species, which typically remain dormant (Goldberg, 1974; Jameson, 1974; Rose, 1981). *S. jarrovi* is also the only ovoviparous species we tested (Guillette, Jones, Fitzgerald, & Smith, 1980), although it is unclear how reproductive mode may affect thermal tolerance in lizards (Wang, Lu, Ma, & Ji, 2013; Wang, Ma, Shao, & Ji, 2017). Despite being the second-largest species in our data set, *S. occidentalis* has a CTmax closer to the smaller sympatric *S. graciosus*

than the larger *S. jarrovi*. Despite phylogeny accounting for variation in CTmax in recent multispecies studies (Leiva et al., 2019; Rubalcaba & Olalla-Tárraga, 2020), differences in CTmax among species in our study are not well explained by phylogenetic relationships, as *S. occidentalis* is most closely related to *S. virgatus* (Grigg & Buckley, 2013; Pyron, Burbrink, & Weins, 2013). Sympatry, or local climate, may have a greater influence on CTmax (Grigg & Buckley, 2013), as CTmax are visually clustered within each sympatric pair (Figure 2). Indeed, in three agamid lizard congeners (*Phrynocephalus*), although CTmax was highest in the smallest species, the largest species had higher CTmax than the intermediate species, presumably due to local habitat differences (Wang et al., 2013). Although altitude varied among populations of the species we sampled, we did not observe species-specific effects of partial pressure of oxygen on CTmax, and altitude appears to have minimal influence on CTmax among

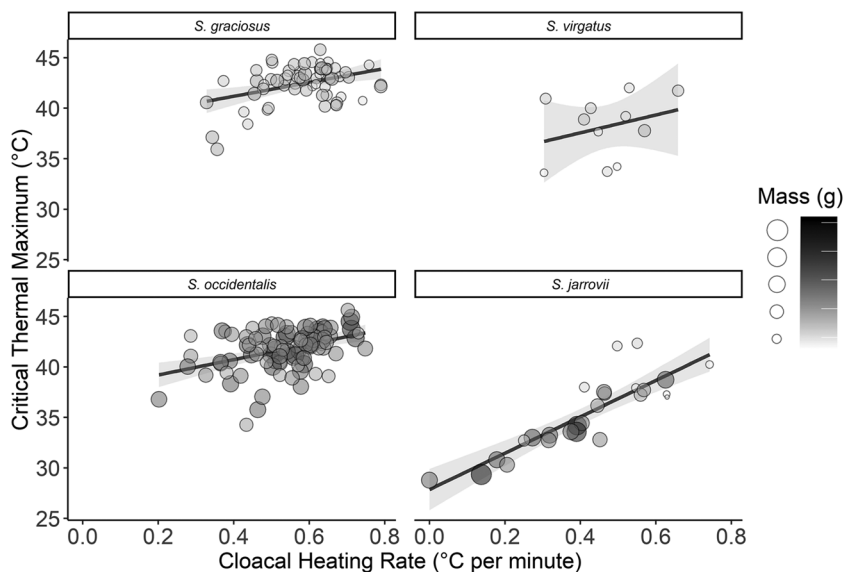


FIGURE 3 The effect of cloacal heating rate on the critical thermal maximum in four species of *Sceloporus* lizards under normoxic conditions. There was a main effect of species, with *Sceloporus jarrovi* having a lower CTmax than all other species; *S. jarrovi* also had the steepest positive slope, highlighting an interaction between cloacal heating rate and species. Heating rate interacted with mass to affect CTmax, with smaller lizards having faster heating rates. Larger masses are represented by larger, darker circles. Shaded area is 95% confidence interval

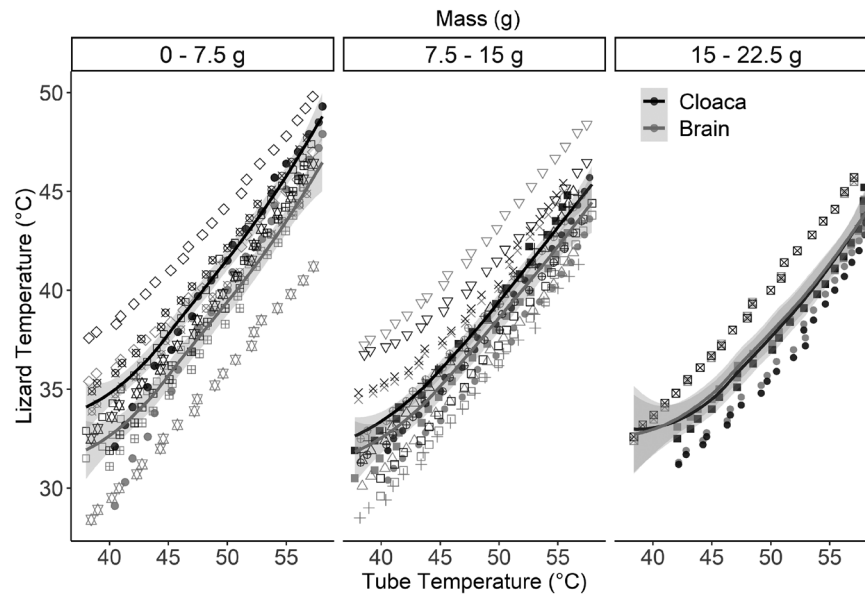


FIGURE 4 The effect of body mass (binned into three groups for easier visualization) on the brain (grey) and cloacal (black) temperatures of living *Sceloporus occidentalis* as they are heated at an ambient (tube) temperature increase of 1°C/min. Individual symbol shapes within each facet represent data from individual lizards at each tube temperature. Smaller lizards heated faster and reached higher brain and cloacal temperatures than larger lizards. In general, cloacal temperatures were higher than brain temperatures at all tube (ambient) temperatures; this is more apparent in smaller lizards, which had greater differences between brain and body temperatures. Shaded area is 95% confidence interval

populations in other species (reviewed in Clusella-Trullas & Chown, 2014; and Huang & Tu, 2008). We observed a positive relationship between mass and CT_{max} in normoxia-subsetted *S. virgatus*; but this could potentially be an artifact of low sample size relative to other species (Table 1). Regardless of inter-specific differences, we generally observed intraspecific negative relationships

between mass and CT_{max}, raising the possibilities that individuals of different body sizes have inherently different CT_{max} due to size-related physiological differences and/or as an artifact of their greater thermal inertia.

Potential physiological reasons for a body size effect on CT_{max} involve metabolic and oxygen delivery constraints. First, total basal metabolic rate increases as body mass increases (Kleiber, 1932), so larger lizards have higher net consumption of oxygen at rest (Roe et al., 2005; Zari, 1991). As lizards are heated, their metabolic rates increase, especially as they often begin scrambling within test chambers and expend extra energy when righting themselves over the course of repeated testing (Camacho & Rusch, 2017), and larger lizards also likely expend more energy while righting. High metabolic rates increase the demand for oxygen. Given that respiratory surface area scales with mass with an exponent of 0.78 in ectotherms and thickness of the respiratory surface is not affected by body mass (Gillooly et al., 2016), applying Fick's law suggests that larger lizards experience lower rates of respiratory diffusion of oxygen than smaller lizards. In some fishes, smaller individuals have higher CT_{max} than larger individuals, likely because smaller fishes can more easily obtain sufficient oxygen at high temperatures (Di Santo & Lobel, 2017; Pörtner, 2010). As aquatic animals, however, fishes are expected to experience much greater selection for hypoxia-tolerance than terrestrial animals like lizards that seldom experience oxygen-limiting conditions. Interestingly, we did not see an interactive effect of mass or species and oxygen partial pressure on CT_{max}, likely because hypoxia had a greater overall influence on reducing CT_{max} (DuBois et al., 2017; Shea et al., 2016). Recent evidence suggests that oxygen transport capacity may evolve to compensate for diffusion

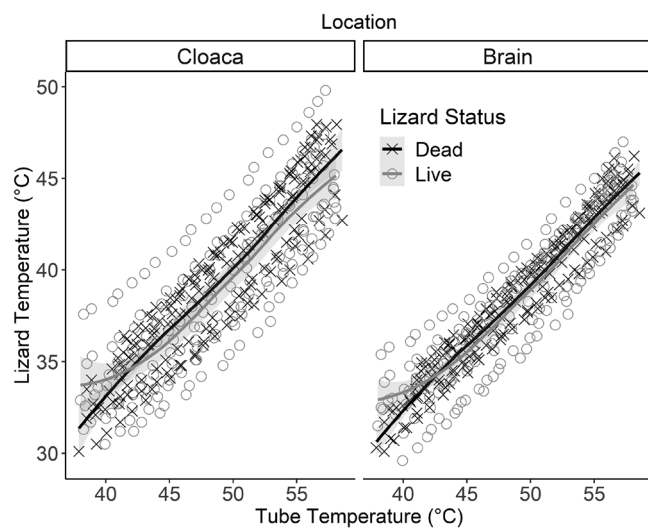


FIGURE 5 The cloacal temperatures and brain temperatures of living and dead *Sceloporus occidentalis* were similar as ambient (tube) temperature increased at 1°C/min, indicating little active physiological regulation of temperatures. Individual symbol shapes within each facet represent data from individual lizards. Shaded area is 95% confidence interval

limitation in larger animals and animals living at lower oxygen partial pressures, and may also increase physiologically as individuals reach higher temperatures and increase metabolic rate (Seibel & Deutsch, 2020). The rate of this compensation is unlikely to occur at our experimental rate of 1°C/min, or with drastic PO₂ changes, thus oxygen limitation may still partially explain why we see loss of neuromuscular coordination, decreased muscle function, and loss of righting response at the CT_{max}, and lower CT_{max} under hypoxic conditions. The higher overall metabolic rate of larger individuals as they are heated could ultimately lead them to reach CT_{max} faster and at lower temperatures than smaller conspecifics, at similar partial pressures.

While at least some of the species and body mass effects we observed on CT_{max} in this study may be explained by size-related differences in metabolic rate or oxygen delivery to tissues, thermal inertia likely plays a major role in the CT_{max} values obtained. While Porter et al. (1973) argued that blood flow should allow rapid equilibration of surface and deep body temperatures in most lizards because of their relatively small size, this has not been tested in many species. Even if universally true, heating rate is affected by body size and can impact CT_{max} (Camacho & Rusch, 2017; Lutterschmidt & Hutchison, 1997; Terblanche, Deere, Clusella-Trullas, Janion, & Chown, 2007). When heating rates are low, animals might have time to acclimate to the increased temperatures, while a high heating rate causes heat-shock, both of which will alter the resulting thermal limits (Lutterschmidt & Hutchison, 1997). In our study, heating rate interacted with mass to affect CT_{max}, with smaller lizards having faster heating rates; both faster heating rates and lower masses independently led to higher CT_{max}. Larger lizards have higher thermal inertia due to their lower surface area to volume ratios, and so their cloacae heat more slowly than smaller lizards. Longer heating durations (due to either a lower starting temperature or a slower heating rate) can result in a lower CT_{max} (Terblanche et al., 2007), therefore at least part of our body size results could be explained by the fact that larger lizards had to be heated for longer to reach their CT_{max} due to thermal inertia.

The high thermal inertia of larger animals lends them greater capacity for regional heterothermy of the body, such that the location of temperature measurement affects the interpretation of the CT_{max}. Due to both regional heterothermy and differences in function, brain temperature could be more relevant as a measure of CT_{max} than cloacal temperature (Hammel, Caldwell, & Abrams, 1967). Body temperature is sensed and integrated in the hypothalamus of the brain, and in amphibians there is evidence that the onset of spasms at high temperatures results from the failure of the central nervous system to coordinate muscle activity (Paulson & Hutchinson, 1987). Although brain temperatures are not feasible to measure in most studies, we sought to characterize the difference between brain and cloacal temperatures as a function of mass to gauge how much the body temperature data we collect may be biased due to brain-cloaca differences. We hypothesized that the cloacal temperatures of larger lizards are less indicative of central (e.g., muscle and nervous tissue) body temperatures than in small

lizards, especially in species which show a great range of size variation. We found that brain temperatures of *S. occidentalis* were consistently lower than cloacal temperatures, that both the cloacae and brains of smaller individuals heated more rapidly than those of larger individuals, but in contrast to our hypothesis, smaller lizards had greater cloacal-brain temperature differentials. This latter pattern matches that observed by Pough and McFarland (1976) for iguanas (*Iguana iguana*, Linnaeus 1758) and is likely due to slower cloacal heating rates in larger individuals due to thermal inertia. Indeed, we found that the size-heating interaction was more pronounced in cloacal rather than brain measurements in *S. occidentalis*. In other words, the brains of both small and large lizards heated at more similar rates than their cloacae; the cloacae of large lizards heated at a slower rate than small lizards due to differences in thermal inertia at the cloaca, potentially due to more tissue insulating the cloaca of larger lizards. Therefore, the observed cloacal-brain temperature differential was smaller for large lizards. Ironically, whereas we had expected thermal inertia of large *S. occidentalis* to be most problematic, our results suggest that cloacal temperatures are actually closer to the more relevant brain temperatures in large lizards than in small lizards (Pough & McFarland, 1976). Though our result is similar to that found for iguanas, which attain larger absolute body sizes than *S. occidentalis* (Pough & McFarland, 1976), the observed effects of thermal inertia at the cloaca and brain are likely relative to the size distribution of the species tested. For example, in lizard species with more extreme size distributions (e.g., *Tupinambis* sp. or *Varanus* sp.), cloacal and brain heating rates may be most similar in a medium size class. Differences in tissue insulation at extreme body sizes (e.g., a neonate vs. large adult *Varanus komodoensis*, Ouwens, 1912) may be so great that brain-cloaca temperatures deviate at both extremes, but insulation and heating rates of brain and cloaca are similar in medium-sized animals. Overall, it will be important to account for body size in future studies on different taxa to account for the effects of thermal inertia on regional heterothermy.

A major caveat is that the lizards in our brain-cloaca study were under deep anesthesia and therefore could not pant to cool themselves via evaporative cooling (Tattersall, Cadena, & Skinner, 2006), a behavior we have previously observed in *S. occidentalis* as they are heated (DuBois et al., 2017; Shea et al., 2016). As lizards are heated, brain temperatures are typically higher than body temperatures to a certain point, and then are lower thereafter (Georges, 1979; Webb, Johnson, & Firth, 1972), likely as a result of panting. Panting reduces brain temperature by 1.3°C and 1.75°C below body temperatures in other lizards (*Anolis carolinensis*, Voigt, 1832, and *Basiliscus basiliscus*, Wagler, 1830, respectively; Crawford, Palomeque, & Barber, 1977), and can keep body temperature and especially brain temperature below high ambient temperatures for extended periods of time in chuckwallas (*Sauromalus ater*, Boulenger, 1885; Crawford, 1972). At high temperatures, iguanas also maintain lower brain temperature than body temperatures (Pough & McFarland, 1976), but this relationship was also true when lizards were dead or when artificial lizards (dialysis bags) were used. We observed some evidence for cooling without panting, potentially due to blood flow and

vasodilation/constriction (Tattersall et al., 2006), as lizards maintained lower brain and cloacal temperatures when alive. However, whether lizards were alive or dead did very little to impact their brain and cloacal heating rates. This shows that vascular control contributes little to brain-body temperature differences during heating when panting is not possible. The observed differences in brain and cloacal temperature therefore result mainly from passive processes like greater heat transfer across the smaller head than the larger body.

5 | CONCLUSION

Our research demonstrates that despite interspecific differences in CT_{max}, variation in intraspecific body size influences measurement and interpretation of CT_{max}. We also show that when panting is not possible, physiological control of brain temperature is minimal. Although higher than brain temperatures, cloacal temperatures can provide a decent approximation of brain temperature (i.e., the source of loss of neuromuscular coordination at CT_{max}) when body size is accounted for. Whether lower observed CT_{max} and heating rates in larger lizards are due to physical processes such as thermal inertia, physiological processes such as oxygen limitation, or an interaction of the two, it will continue to be important to account for body size differences in studies of ectotherm thermal physiology if these processes are to be better understood.

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DATA AVAILABILITY STATEMENT

Data set for Body size impacts critical thermal maximum measurements in lizards is available at Dryad: <https://doi.org/10.5061/dryad.18931zcv8>.

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