Effects of oxygen on responses to heating in two lizard species sampled along an elevational gradient

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ABSTRACT

Thermal tolerance is an important variable in predictive models about the effects of global climate change on species distributions, yet the physiological mechanisms responsible for reduced performance at high temperatures in air-breathing vertebrates are not clear. We conducted an experiment to examine how oxygen affects three variables exhibited by ectotherms as they heat—gaping threshold, panting threshold, and loss of righting response (the latter indicating the critical thermal maximum)—in two lizard species along an elevational (and therefore environmental oxygen partial pressure) gradient. Oxygen partial pressure did not impact these variables in either species. We also exposed lizards at each elevation to severely hypoxic gas to evaluate their responses to hypoxia. Severely low oxygen partial pressure treatments significantly reduced the gaping threshold, panting threshold, and critical thermal maximum. Further, under these extreme hypoxic conditions, these variables were strongly and positively related to partial pressure of oxygen. In an elevation where both species overlapped, the thermal tolerance of the high elevation species was less affected by hypoxia than that of the low elevation species, suggesting the high elevation species may be adapted to lower oxygen partial pressures. In the high elevation species, female lizards had higher thermal tolerance than males. Our data suggest that oxygen impacts the thermal tolerance of lizards, but only under severely hypoxic conditions, possibly as a result of hypoxia-induced anapyrexia.

1. Introduction

Global climate change is expected to increase mean annual global temperatures by at least 0.8 °C, possibly more than 2 °C by 2050 (Collins et al., 2013). This change in the global climate will result in a massive restructuring of ecosystems worldwide and could lead to widespread species extinction (Thomas et al., 2004; Parmesan, 2006; Sinervo et al., 2010; Brusch et al., 2016). For this reason, concern is mounting for the longevity of many thermally sensitive species. Ectotherms are at particular risk because of the tight relationship between temperature and rates of physiological processes (Dawson, 1975; Huey and Stevenson, 1979). An increase in global temperatures can push many habitats outside of the physiological tolerance ranges of ectotherms (Brusch et al., 2016), leading to changes in growth, maintenance, reproduction, and survival (Bennett, 1980; Cloudsley-Thompson, 1999; Angilletta et al., 2002; Sinervo et al., 2010). Although thermoregulatory shuttling behavior may ameliorate some of these negative effects (Adolph, 1990; Buckley et al., 2015; Levy et al., 2015; Pincebourde et al., 2016), warming of the environment beyond

physiological tolerance ranges is expected to severely impact populations (Sinervo et al., 2010; Brusch et al., 2016). To better understand how climate change will affect terrestrial organisms and their habitats, we must investigate the thermal tolerances of ectothermic and thermally sensitive species from diverse and integrative perspectives.

A commonly used variable of thermal tolerance is the critical thermal maximum (CTMax). CTMax is defined as the maximum body temperature at which an organism is able to sustain basic vital physiological functions, with exposure to higher temperatures leading to death (Cowles and Bogert, 1944). Furthermore, studies have shown that the observable response to CTMax—loss of coordinated muscle function and righting response followed by the onset of spasms—occurs in all reptilian taxa studied (reviewed in Lutterschmidt and Hutchison, 1997b). Even with a long history of studies on CTMax, the mechanisms underlying the loss of righting response and onset of spasms remain poorly understood (Schulte, 2015). The oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis suggests that a decline in performance occurs due to the lack of oxygen delivery to body tissues at higher temperatures (Frederich and Pörtner, 2000). In recent years,

Table 1
Summary of Lizard Data and Experimental Design.

Site	Elevation (m)	Species	Treatment	P_{O2} (mmHg)	N	SVL (mm)	Mass (g)
Hospital Flats Campground	900	*S. occidentalis*	normoxia	144.0	12 (6M, 6F)	72.3 ± 1.6	15.0 ± 1.1
			hypoxia	41.2	10 (4M, 6F)	69.3 ± 6.6	15.7 ± 1.8
Kern River Trailhead	1475	*S. occidentalis*	normoxia	135.0	12 (4M, 8F)	66.1 ± 1.8	11.5 ± 0.9
			hypoxia	38.6	6 (2M, 4F)	67.8 ± 13.1	12.5 ± 2.2
Poison Creek	2100	*S. occidentalis*	normoxia	125.4	5 (3M, 2F)	69.8 ± 13.3	11.9 ± 2.3
			hypoxia	35.8	6 (3M, 3F)	73.8 ± 14.4	12.5 ± 2.5
		S. graciosus	normoxia	125.4	11 (4M, 7F)	55.1 ± 1.0	8.8 ± 2.1
			hypoxia	35.8	11 (6M, 5F)	57 ± 1.0	6.8 ± 0.4
Round Meadow	2680	*S. graciosus*	normoxia	117.2	11 (3M, 8F)	55.2 ± 0.8	6.4 ± 0.3
			hypoxia	33.5	11 (7M, 4F)	57.7 ± 0.9	7.0 ± 0.4

Note: Summary data on *Sceloporus occidentalis* and *S. graciosus* tested at four different sites along an elevational gradient in this study. The sample size (N) represents the number of lizards brought to the critical thermal maximum (CTMax). In some case, lizards did not gape or pant before attaining CTMax, so sample sizes for those tests may be smaller (see text).

this hypothesis has received a lot of attention (reviewed in Verberk et al., 2016). Several studies have shown that oxygen can be a limiting factor on thermal tolerances in water-breathing animals (Pörtner, 2001; Pörtner and Knust, 2007; Verberk and Bilton, 2011; Verberk and Calosi, 2012; but see Clark et al., 2013; Ern et al., 2014; Norin et al., 2014) and in air-breathing organisms (Verberk and Bilton, 2013, 2015; Verberk et al., 2013; Koopman et al., 2016; Shea et al., 2016), while most studies have not found support for OCLTT hypothesis in air-breathers, potentially because oxygen is so easily obtained (Klok et al., 2004; Stevens et al., 2010; McCue and De Los, 2013; Boardman and Terblanche, 2015). Fewer studies have tested this hypothesis in air-breathing vertebrates, and most have failed to support it (Seebacher and Franklin, 2011; Overgaard et al., 2012; Fobian et al., 2014; but see Smith et al., 2015; Shea et al., 2016).

Whereas the concept of oxygen limitation of air-breathing vertebrates has only recently received attention, there is a large literature showing that severe hypoxia can dramatically reduce the temperature set point of ectotherms, a phenomenon termed hypoxia-induced anapyrexia (Hicks and Wood, 1985; Dupré et al., 1986; Wood, 1991). Ectotherms exhibit a series of physiological and behavioral responses to hypoxia (Hicks and Wang, 2004), including initiation of panting at lower temperatures and choosing lower temperatures in a gradient, presumably to mitigate the metabolic costs of high body temperature under hypoxic conditions. In response to heating or while attempting to avoid heating beyond the preferred body temperature, many ectotherms exhibit gaping (opening of the mouth) and panting, potentially to enhance evaporative cooling (Heatwole et al., 1973); under hypoxic conditions, these behaviors may occur at lower temperatures to help the animal maintain a lower body temperature and therefore conserve oxygen (Wood and Gonzales, 1996). Although the temperatures at which animals exhibit gaping and panting behaviors do not necessarily reflect thermal tolerance in the way that the CTMax does, they represent robust and quantifiable behavioral responses to heating. While studies of hypoxia-induced anapyrexia do not directly test the OCLTT hypothesis because they do not demonstrate that physiological demand for oxygen outpaces the supply under normoxic circumstances, it is useful to compare the gaping threshold, panting threshold, and CTMax of animals under severe hypoxia to those observed during normoxia. Such comparisons may help explore what role, if any, oxygen plays in determining thermal tolerance. Further, they may reveal whether the OCLTT hypothesis is an appropriate model to apply to an ectothermic, air-breathing vertebrate. Furthermore, the vast majority of thermal tolerance studies in ectotherms, whether conducted under normoxic or hypoxic conditions, take place in captivity using acclimated laboratory animals. Given that thermal tolerance variables like the CTMax may be impacted by

acclimation (Spellerberg, 1972), it is desirable to conduct studies on animals in a natural setting shortly after capture.

We investigated the effect of inhaled oxygen partial pressures on the thermal tolerance of two species of lizards, *Sceloporus occidentalis* (western fence lizard) and *S. graciosus* (western sagebrush lizard), which are ideal species for thermal tolerance experiments due to the wealth of studies examining sensitivity to high temperatures in multiple lizard species (Cowles and Bogert, 1944; Brattstrom, 1965; Lutterschmidt and Hutchison, 1997b; Angilletta et al., 2002;). We conducted a field study examining the thermal tolerance of the two species across an elevational gradient, where ambient oxygen concentrations (e.g., environmental oxygen partial pressures, P_{O2}) vary. This experiment had several objectives. First, we examined how severe hypoxia impacts the gaping threshold, panting threshold, and CTMax of lizards along the elevational gradient to examine hypoxia-induced anapyrexia in a natural setting. Second, we took advantage of naturally occurring differences in P_{O2} to conduct an indirect test of the OCLTT hypothesis. If oxygen limits thermal tolerance, we predict that lizards will exhibit reduced thermal tolerance at higher elevations, where oxygen is lower. Third, we compared the thermal tolerance variables of the two species at the one elevation in which they overlapped, and of males and females of each species to examine potential species and sex differences in thermal tolerance and response to hypoxia.

2. Materials and methods

2.1. Study site and species

In the Sierra Nevada mountains of California, *S. occidentalis* is common at lower elevations and *S. graciosus* appears at higher elevations, with a narrow range of overlap. Lizards were sampled along an elevational gradient in the Sequoia National Forest in the Southern Sierra Nevada in the month of July. Four sampling locations were chosen (Table 1). Lizards were captured by hand or with a noose and stored in numbered tube socks for transport. Sample sizes are shown in Table 1. Mass (± 0.5 g), snout vent length (SVL, ± 1 cm), tail length (± 1 cm), time of capture, sex, and reproductive state (females only; carrying eggs=gravid, or not) were recorded for each lizard. Lizards were tested for gaping and panting thresholds and CTMax (see below) within 300 m of their capture location on the same day of their capture. Each lizard was tested only once. All procedures were approved by the Institutional Animal Care and Use Committee of the California Polytechnic State University (protocol #1506) and the California Department of Fish and Wildlife (scientific collecting permit #13265).
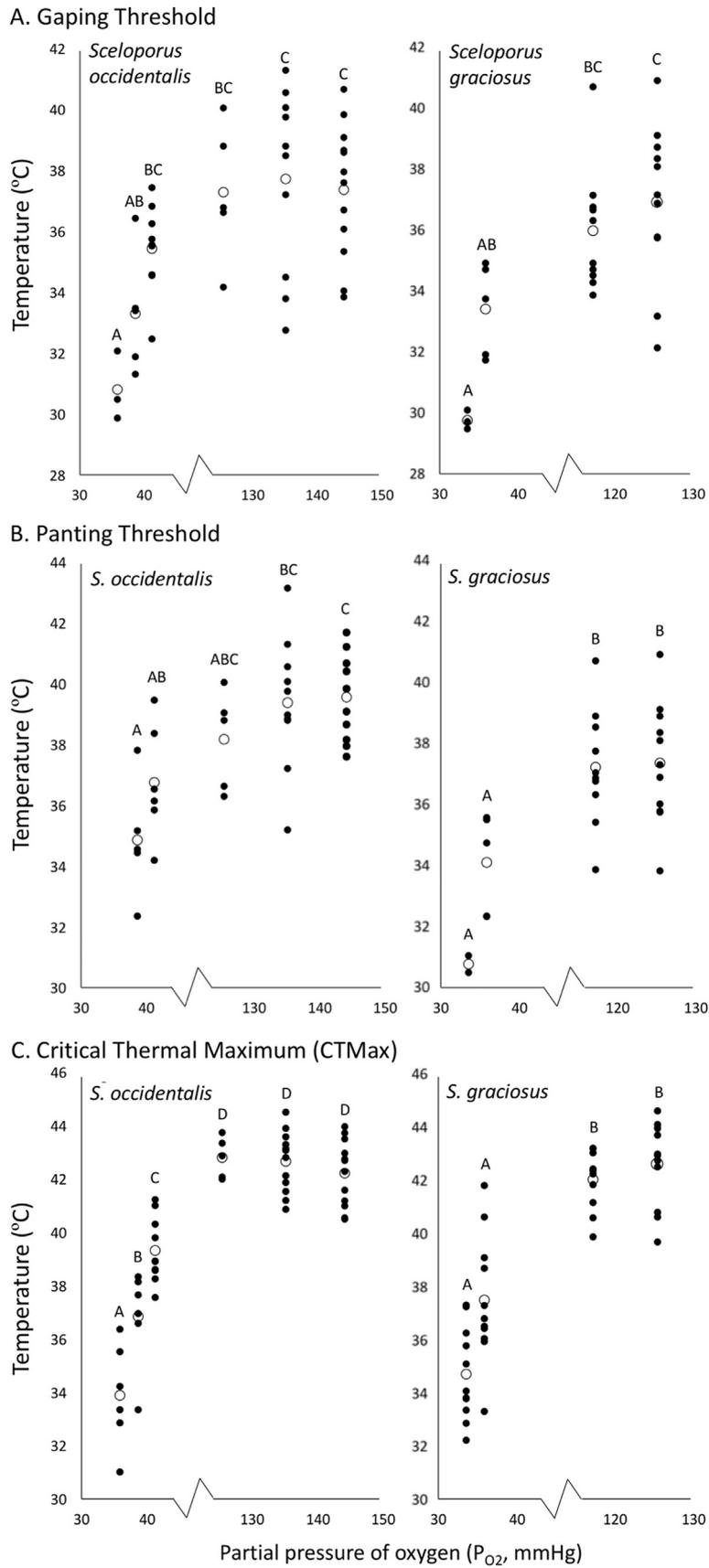
Fig. 1. Low oxygen partial pressures progressively reduced the (A) gaping threshold, (B) panting threshold, and (C) loss of righting response designated as critical thermal maximum (CTMax) in *Sceloporus occidentalis* (left panels) and *S. graciosus* (right panels) sampled along an elevational gradient. Solid circles represent single data points, and open circles represent the average gaping threshold of lizards in that treatment. Within each species, groups with different letters were significantly different.

2.2. Measurements of thermal tolerance

All thermal tolerance tests were carried out using a field-portable, multiplex apparatus, the Controlled Reptile Oxygen and Climate System (CROCS), which allowed us to heat up to six lizards at once with gases of varying oxygen concentrations (for detailed description of CROCS, see Shea et al., 2016). Briefly, air from a scuba tank or compressor flows through a heating system and is delivered to six clear, silicone chambers, each containing a lizard.

Temperature probes consist of resistance temperature detectors (Honeywell platinum RTD), coated with waterproof and highly thermally conductive epoxy (KONA 870 FTLV-DP), and tested against a standard thermometer. Probes are inserted 1 cm into lizards' cloacae and secured using medical tape to monitor body temperature during testing. Chambers were heated at a rate of 1 °C per minute (Lutterschmidt and Hutchison, 1997b). Lizards were randomly assigned to be treated with either normoxic air (21% oxygen, consisting of atmospheric air supplied via an air compressor) or hypoxic air (6% oxygen, 94% nitrogen, from 80 ft^3 aluminum compressed gas cylinders). As a result of the variation in elevation, these treatments resulted in lizards being exposed to one of a spectrum of eight P_{O2} ranging from 33 to 144 mmHg.

As the lizards were heated, at least two observers monitored them and recorded the following three variables: gaping threshold (the body temperature at which lizards first opened their mouths), panting threshold (the body temperature at which lizards exhibited rapid and sustained chest compressions with open mouths), and the loss of righting response (the point at which lizards failed to right themselves after being flipped on their backs). We chose the loss of righting response instead of onset of spasms (Lutterschmidt and Hutchison, 1997a) as a proxy for CTMax because lizards failed to show muscular spasms before reaching the upper lethal temperature in previously conducted pilot studies (Shea et al., 2016). The cylindrical test chambers of CROCS allowed us to rapidly rotate test subjects to evaluate the righting response. Following gaping, lizards were flipped onto their backs about 4–5 times per minute until they reached a temperature at which they could no longer right themselves. We use the loss of righting response as a proxy for the CTMax (Cowles and Bogert, 1944; Lutterschmidt and Hutchison, 1997a). Observers were blind to the actual T_b of lizards during testing, which was uploaded onto a computer managed by a third observer. This person recorded the T_b once the other two observers agreed the righting response was lost, hereafter referred to as the CTMax. After attaining CTMax, lizards were immediately removed from CROCS, allowed to recover, and released at the exact locations at which they were captured.

2.3. Data analysis

All data analyses were performed in JMP version 11.0. Separate ANCOVA models were constructed for each variable (gaping threshold, panting threshold, and CTMax) and for each species. Each model was constructed with P_{O2}, sex, SVL, mass, gravidity, time of day, and time elapsed since capture. Tukey HSD tests were used to determine whether thermal tolerance variables were significantly different at each P_{O2}. In addition to the separate species models, we compared the thermal tolerance variables of the two species at the single elevation at which both were present and received the same oxygen treatment (Poison Creek, 2100 m, see Table 1) using an ANCOVA with species and treatment (hypoxia or normoxia instead of P_{O2} because this test was at a single elevation) as factors along with sex, SVL, mass, gravidity, time of day, and time elapsed since capture. Only significant factors, covariates, and interactions were included in final models. For the common elevation analysis, because *S. occidentalis* is larger than *S. graciosus* and potentially has higher thermal inertia, we also investigated whether body size affected the heat exposure time (e.g., amount of time from initiation of heating to CTMax) by running models with

treatment, species, and either SVL or mass, and their interactions, on the response variable of heat exposure time. CTMax data were transformed to meet assumptions of normality using a box-cox transformation. All data met the assumption of homogeneity of variance. For all analyses, final models are reported in the Results and included only significant variables. Alpha was set at 0.05.

3. Results

Some lizards did not exhibit gaping or panting behaviors prior to reaching the CTMax, resulting in different degrees of freedom among the models. For both species, lower P_{O2} progressively reduced the gaping threshold (*S. occidentalis*: $F_{5,38}$ =7.11, p < 0.0001; *S. graciosus*: $F_{3,25}$ =10.54, p < 0.0001; Fig. 1A), the panting threshold ($F_{4,32}$ =7.78, p=0.0002; *S. graciosus*: $F_{3,24}$ =10.24, p=0.0002; Fig. 1B), and the CTMax (*S. occidentalis*: $F_{5,45}$ =51.11, p < 0.0001; *S. graciosus*: $F_{4,39}$ =46.66, p < 0.0001; Fig. 1C). However, Tukey posthoc tests showed that the effect of P_{O2} was exclusively due to the severe hypoxia treatment, where the three variables were strongly affected by very low P_{O2} (Fig. 1). Under normoxic conditions, these variables were not affected by P_{O2}. For *S. graciosus* only, there was a significant effect of sex on CTMax, with females achieving a higher CTMax than males ($F_{4,39}$ =4.83, p=0.034; Fig. 2).

At the single site where *S. occidentalis* and *S. graciosus* were both present, hypoxic treatment significantly reduced the gaping threshold ($F_{3,20}$ =24.32, p < 0.0001), but this effect was consistent for both species (Species: $F_{3,20}$ =1.27, p=0.27; Treatment by species interaction: $F_{3,20}$ =2.12, p=0.16). The effect of species on panting threshold could not be evaluated at this elevation because no *S. occidentalis* panted before attaining the CTMax. At this site, there was a significant interaction between species and treatment on CTMax ($F_{5,27}$ =4.76, p=0.0068). Tukey posthoc tests showed that the CTMax of the two species did not differ significantly under normoxic conditions, but that *S. occidentalis* has a significantly lower CTMax than *S. graciosus* under hypoxic conditions (Fig. 3). The differential effect of hypoxia on the two species was not due to differences in thermal inertia, because hypoxia did not differentially affect heat exposure time of different-sized lizards (SVL by treatment interaction: $F_{4,32}$ =2.28, p=0.14; mass by treatment
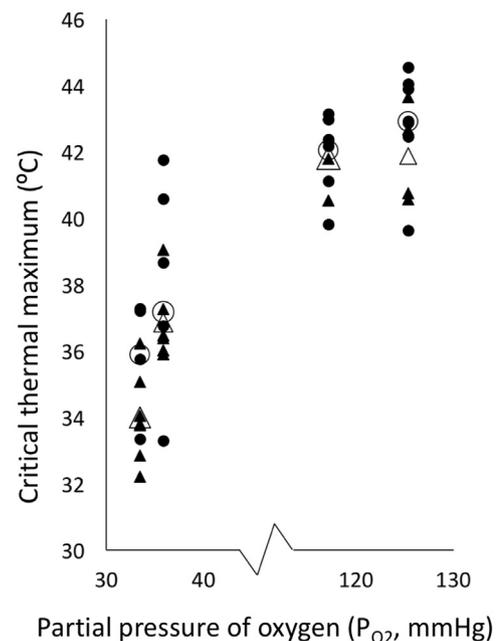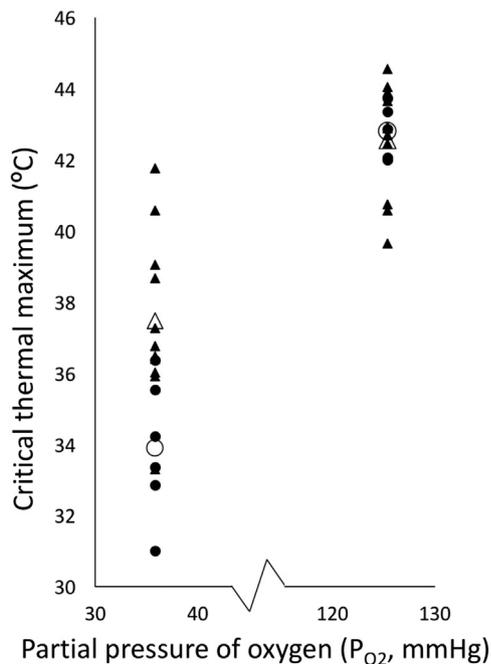

Fig. 2. Effect of oxygen partial pressure on the critical thermal maximum (CTMax) in male (triangles) and female (circles) *Sceloporus graciosus*. Mean values for each sex are represented by open symbols. Females had significantly higher CTMax than males ($F_{4,39}$ =4.83, p=0.034).

Fig. 3. Effect of oxygen treatment (hypoxic, 6% O_2, or normoxic, 21% O_2) on the critical thermal maximum (CTMax) in *Sceloporus occidentalis* (circles) and *S. graciosus* (triangles) at a 2100 m site where they occur sympatrically. Mean values for each species are represented by open symbols. There was a significant interaction between treatment and species, where hypoxia led to a greater reduction in CTMax in *S. occidentalis* than *S. graciosus* ($F_{5,27}$ =4.76, p=0.0068).

interaction: $F_{4,32}$ =0.63, p=0.43). Sex was also a significant factor, with females achieving a higher CTMax than males ($F_{5,27}$ =4.76, p=0.038). Although the sex difference in CTMax at this site qualitatively appears to more pronounced in *S. graciosus* than *S. occidentalis* (Fig. 2), sex did not significantly interact with species or any other variables.

4. Discussion

Our study took advantage of an elevational transect that created a gradient of P_{O2} to examine the relationship between oxygen concentration and gaping threshold, panting threshold, or CTmax. We found that P_{O2} did not affect these variables in lizards under normoxic conditions, but that hypoxia dramatically reduced all three in a dose-dependent fashion. Importantly, all data were collected in situ, within hours of capturing the animals, and with a device designed to maximize accuracy, so the data represent highly relevant and accurate values.

The fact that varying P_{O2} had no impact on thermal tolerance under normoxic conditions does not provide support for the OCLTT hypothesis as a mechanism behind thermal tolerance in lizard populations. If oxygen had been a limiting factor as the lizards were heated and their oxygen demand intensified, we would expect that lizards at higher elevations (and therefore lower P_{O2}) would exhibit lower thermal tolerance than lizards at lower elevations. The natural reduction in P_{O2} with elevation may not be severe enough to reduce oxygen saturation of hemoglobin, such that metabolic function is not compromised by the small change in oxygen. Also, lizards at different elevations may be adapted to different temperatures, which could impact the data collected. It is important to note that our study provided an indirect test of the OCLTT hypothesis, while a more direct test would necessitate showing a drop in blood oxygen and/or metabolic rate or an increase in anaerobic metabolism as the CTMax approaches (Overgaard et al., 2012; Fobian et al., 2014; Verberk et al., 2016). Unfortunately, we did not have the ability to collect such data in a remote field setting. Nonetheless, the lack of an impact of P_{O2} on thermal tolerance under naturally occurring conditions suggests that

oxygen likely is not a major component involved in the physiological response to high temperatures. Of course, lizards at higher elevations could be adapted to the lower oxygen availability such that they exhibit similar thermal tolerance to low elevation lizards in spite of the difference in oxygen. With regard to air-breathing organisms, oxygen is more easily obtained and therefore less likely to be a limiting factor in thermal tolerance. Studies on air-breathers have focused mainly on arthropods, and support is mixed (Klok et al., 2004; Stevens et al., 2010; Bjelde et al., 2015; Boardman and Terblanche, 2015; Verberk and Bilton, 2015; Koopman et al., 2016; Verberk et al., 2016). Very few studies have experimentally tested the OCLTT hypothesis in air-breathing vertebrates, and most have not found support for it (Seebacher and Franklin, 2011; Overgaard et al., 2012; Fobian et al., 2014).

Whereas P_{O2} did not impact thermal tolerance under normoxic conditions, under severely hypoxic conditions the oxygen levels are tightly linked to the gaping and panting thresholds and the CTMax. Exposure to severe hypoxia has been shown to have similar effects in a number of other studies, including reduced gaping thresholds (Dupre et al., 1986; Tattersall and Gerlach, 2005; Shea et al., 2016), reduced CTMax (Shea et al., 2016), and reduced survival of embryos (Smith et al., 2015). The reduced gaping and panting thresholds may represent hypoxia-induced anapyrexia, where the lizards experience a reduced temperature set point and attempt to reduce body temperature at a lower threshold when exposed to the severe metabolic challenge of hypoxia (Hicks and Wood, 1985; Dupré et al., 1986; Wood, 1991). This interpretation seems less likely when considering the loss of righting response (CTMax), which at its core is a measure of a loss of locomotory function. It is possible that lizards lost their righting response at such low temperatures under severe hypoxia in this study may be more of a behavioral response than an oxygen-limited, physiological response. For example, it is possible that the hypoxic stimulus caused them to stop righting themselves in order to reduce oxygen-demanding locomotion at high temperatures; however, this interpretation seems unlikely. We could be tempted to interpret the reduction in CTMax under hypoxic conditions as support for the OCLTT hypothesis (Shea et al., 2016). However, ecologically relevant predictions of the OCLTT hypothesis require measuring metabolic rate under natural conditions, not under conditions of severe hypoxia that lizards are unlikely to experience in nature. Again, without data showing how the various P_{O2} treatments impacted blood oxygen levels, aerobic metabolic rates, aerobic scope, and anaerobic metabolism, it is difficult to evaluate the extent to which hypoxia physiologically limited the performance of the lizards as opposed to initiating a behavioral anapyrexic response.

Species and sex differences in thermal tolerance are rarely investigated, especially from the perspective of oxygen availability. Since *S. graciosus* and *S. occidentalis* were sympatric at only one of the testing locations, we have limited data on interspecific variation in thermal tolerance. The treatment by species interaction for CTMax suggests that *S. graciosus* is better able to handle hypoxic conditions, thus achieving a higher CTMax, than *S. occidentalis*. This may be indicative of adaptation to high elevations, since *S. graciosus* is only found at high elevations where P_{O2} is naturally low. Indeed, Vinegar and Hillyard (1972) showed that the oxygen carrying capacity in *S. occidentalis* increased as elevation increased. This could underlie the mechanism by which the two species react differently to low oxygen levels. Alternatively, the difference between species could also have arisen from their differences in body size (Table 1). However, we showed that heat exposure time was not affected by body size under hypoxic conditions, so differences in thermal inertia due to body size are not responsible for the result (Rezende et al., 2014). Body size is negatively correlated with oxygen affinity in snakes (Pough, 1977), and if this holds true for *Sceloporus* lizards then a lower oxygen affinity in *S. occidentalis* might account for the lower CTMax compared to *S. graciosus*. To further investigate this, future studies might capture

animals from different elevations and bring them to a common elevation for testing such that they are treated with the same P_{O_2}. This would control for the differences in P_{O_2} that result from testing lizards at their own capture sites, and would show whether *S. graciosus* is in fact better adapted to high elevations than *S. occidentalis* independent of body size. In addition, such an experimental design could help elucidate whether thermal adaptation in lizard populations inhabiting different elevations is responsible for observed differences in thermal tolerance in response to hypoxic conditions.

For the most part, we did not detect differences between the sexes in thermal tolerance, with one exception: female *S. graciosus* attained a higher CTMax than males. While thermal tolerance may be a species-specific trait, it also may be a dynamic variable that may reflect the physiological state and/or recent thermal history of lizards in the field (Hutchison and Dupre, 1992). For example, many of the female lizards in our study were gravid, and so they may have been engaging in more basking activity during the study than males, especially at higher elevations where temperatures are cooler. Bashey and Dunham (1997) demonstrated that lizards at high elevations face different thermo-regulatory challenges than those at low elevations, and will spend more time basking due to the lower average temperatures associated with high elevation. This difference in thermoregulatory behavior perhaps reveals an effect of sex within *S. graciosus* that does not manifest or is not selected for in *S. occidentalis*. Sievert and Hutchison (1989) found that when placed in a thermal gradient with artificial lighting, female Eastern collared lizards (*Crotaphytus collaris*) exhibited higher pre-ferred body temperatures than males during the summer, but that differences between the sexes might change depending on the time of day, season, and the position of the light source relative to the heater. In another study on *S. occidentalis* (conducted at sea level), Shea et al. (2016) found no sex differences in gaping threshold or CTMax but did observe that females had higher panting thresholds than males. In contrast, Tattersall and Gerlach (2005) found that female bearded dragons (*Pogona vitticeps*) had lower gaping thresholds than males both in normoxic and hypoxic conditions, while Heatwole et al. (1973) found no sex differences in panting threshold in jacky dragons (*Amphibolurus muricatus*). Future studies that examine the sex differences in metabolic rate, hydration, hematocrit, or muscle mass may help us explain the observed effect of sex on thermal tolerance of *S. graciosus*.

Global climate change is predicted to contribute to the extinction of at least 18% of all species by 2050 (Thomas et al., 2004), including up to 20% of lizard species (Sinervo et al., 2010). For ectothermic organisms, a small change in the daily temperature regime can severely limit activity time and have dramatic impacts on the population (Grant and Dunham, 1988; Brusch et al., 2016). In addition, species at lower elevations will have access to fewer thermally acceptable microhabitats than their high elevation counterparts, simply due to the higher average temperatures associated with low elevation (Bashey and Dunham, 1997). Lower elevation species might accommodate for such changes in their environment by expanding their range into higher elevations, in search of cooler climates (Walther et al., 2002; Massot et al., 2008; Sinervo et al., 2010). As average temperatures increase, high elevation species may eventually be unable to continue their range shift, and be at higher risk of extinction as species from lower elevations invade. Understanding the mechanisms behind the thermal limits of terrestrial organisms will help us better understand the effects global climate change will have on their distribution, and allow us to predict what species and populations are most at-risk for extinction.

Acknowledgements

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