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ORIGINAL ARTICLE

Oxygen supply did not affect how lizards responded to thermal stress

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Abstract

Zoologists rely on mechanistic niche models of behavioral thermoregulation to understand how animals respond to climate change. These models predict that species will need to disperse to higher altitudes to persist in a warmer world. However, thermal stress and, thus, thermoregulatory behavior may depend on atmospheric oxygen as well as environmental temperatures. Severe hypoxia causes animals to prefer lower body temperatures, which could be interpreted as evidence that oxygen supply limits heat tolerance. Such a constraint could prevent animals from successfully dispersing to high elevations during climate change. Still, an effect of oxygen supply on preferred body temperature has only been observed when oxygen concentrations fall far below levels experienced in nature. To see whether animals perceive greater thermal stress at an ecologically relevant level of hypoxia, we studied the thermoregulatory behavior of lizards (*Sceloporus tristichus*) exposed to oxygen concentrations of 13% and 21% (equivalent to PO_2 at 4000 m and 0 m, respectively). In addition, we exposed lizards to 29% oxygen to see whether they would accept a higher body temperature at hyperoxia than at normoxia. At each oxygen level, we measured a behavioral response to heat stress known as the voluntary thermal maximum: the temperature at which a warming animal sought a cool refuge. Oxygen concentration had no discernable effect on the voluntary thermal maximum, suggesting that lizards experience thermal stress similarly at all 3 levels of oxygen (13%, 12% and 29%). Future research should focus on thermoregulatory behaviors under ecologically relevant levels of hypoxia.

Key words: hypoxia, *Sceloporus*, stress, temperature, thermoregulation

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INTRODUCTION

Zoologists rely increasingly on mechanistic models of the niche to understand how animals respond to climate change (Kearney & Porter 2009; Kearney *et al*. 2010a; Buckley & Kingsolver 2012). These models describe the thermoregulatory behavior of an animal in a given climate and determine whether this behavior confers the body temperatures needed to survive and reproduce. Given the climate of a region, one can use a mechanistic niche model to predict the geographic range of a species (Kearney *et al.* 2008; Buckley *et al.* 2010; Chapman *et al.* 2017). In principle, then, such models can tell us where species can live in future climates (Kearney *et al.* 2010b; Chapman *et al.* 2017; Petric *et al.* 2017). However, researchers recognize that current models still contain glaring oversimplifications that likely preclude accurate predictions for the distant future (Sinclair *et al.* 2016). Inaccuracies result from averaging environmental temperatures (Vasseur *et al.* 2014; Camacho *et al.* 2015), ignoring biotic interactions (Angert *et al.* 2013) and omitting life stages (Levy *et al.* 2015). Until these issues are resolved, mechanistic niche models are more of a tool for guiding research than a key to predicting impacts.

Here, we focus on another oversimplification of niche models revealed by studies of heat tolerance. An animal can overheat when high temperatures cause tissues to require more oxygen than the respiratory and circulatory systems can supply, and increasing reliance on anaerobic metabolism eventually causes death (Hicks & Wood 1985; Pörtner 2001, 2002a,b). Perhaps for this reason, many ectothermic animals choose lower temperatures when exposed to a hypoxic environment (reviewed by Wood & Gonzales 1996; Hicks & Wang 2004). For example, reptiles and amphibians exposed to oxygen concentrations below 10% preferred lower body temperatures than those exposed to normoxia (Hicks & Wood 1985; Branco *et al.* 1993; Bicego-Nahas *et al.* 2001; Cadena & Tattersall 2010; He *et al.* 2013; Li *et al.* 2016). This shift in thermoregulatory behavior, referred to as behavioral hypothermia or hypoxic anapyrexia, involves sensory neurons that respond to the deficit in circulating oxygen (Tamaki & Nakayama 1987; Steiner & Branco 2002) and chemical messengers that mediate a change in body temperature (Steiner & Branco 2001, 2002). To facilitate cooling, some lizards even lighten their body color under hypoxia, reducing heating by solar radiation (de Velasco & Tattersall 2008). Behavioral hypothermia can prevent an abnormally low concentration of oxygen or energetic molecules in the blood. For example, experimental reduction of ATP caused toads to choose lower temperatures (Branco & Malvin 1996). If animals thermoregulate differently when exposed to hypoxia, atmospheric oxygen and environmental temperature could interact to limit the potential distribution of a species.

The interactive effect of oxygen and temperature becomes especially relevant considering that climate warming shifts the elevational ranges of species. As climates have warmed, many species have moved up mountains (Parmesan & Yohe 2003) because air cools by an average of 0.65 °C for every 100 m of elevation (Dillon *et al.* 2006). As long as predators, competitors and other factors remain within tolerable limits, dispersing upward provides a way for threatened species to persist. Nevertheless, biologists overestimate the potential for dispersal along mountains by focusing exclusively on temperature. Ascending in elevation not only cools the environment but also reduces atmospheric pressure, increases solar radiation and alters vegetation cover. These changes interact to create a unique operative environment, which may lie outside the fundamental niche of a species. In particular, a low partial pressure of oxygen (PO_2) at high elevation could exacerbate the stress of warming by reducing an animal's lethal temperature. Consequently, the elevational limit of a species may depend on the combined stresses of radiative load and oxygen supply. Even if a species persists at high elevation, its rate of growth and reproduction might suffer if low PO_2 prevents activity at high temperatures.

Still, at least 2 factors prevent us from inferring the ecological significance of behavioral hypothermia during hypoxia. First, this response occurs at oxygen concentrations far below levels commonly experienced by animals in nature. By comparison, most ectothermic animals experience partial pressures of oxygen between 13 and 21 kPa, which is equivalent to 13% to 21% oxygen at sea level. Second, animals rarely permit their body temperatures to approach lethal limits when given a choice. By choosing cool locations or evaporating body water, animals avoid reaching temperatures that impair physiological functions (Martin & Huey 2008). An animal overheats only after exhausting its behavioral and physiological options for cooling. Thus, researchers have focused on voluntary warming under severe hypoxia, whereas nature imposes involuntary warming under modest hypoxia. We must understand how animals respond to extreme heating under realistic levels of hypoxia to know whether these conditions will constrain their geographic distribution.

We measured the voluntary thermal maximum of plateau fence lizards (*Sceloporus tristichus* Cope, 1875) exposed to oxygen levels ranging from hypoxia to hyperoxia. The voluntary thermal maximum equals the temperature at which a warming animal seeks refuge from heat (Camacho *et al.* 2018). This parameter reflects an animal's perception of thermal stress and can be used to model thermal constraints on activity time and geographic distribution (Kearney & Predavec 2000; Buckley *et al.* 2015; Levy *et al.* 2016). Our experiment focused on a species that ranges from sea level to elevations of 3050 m. At the highest elevation in this current range, low air pressure impairs the diffusion of oxygen, analogous to breathing air with 15% oxygen at sea level; however, in the coming century, the elevational range of this species could shift to the point where some individuals experience effective concentrations of oxygen as low as 13% (Buckley 2010; Buckley *et al.* 2010). We sought to determine whether this level of hypoxia would alter the thermoregulatory behavior of lizards and, therefore, impair the ability of *S. tristichus* to shift its range to higher altitudes. To confirm that a shift in behavior was mediated by oxygen supply, we also measured the voluntary thermal maxima of lizards under hyperoxia. If oxygen limits the thermal tolerance of these lizards, we expected individuals exposed to lower levels of oxygen to seek refuge from heat at lower body temperatures.

MATERIALS AND METHODS

In May 2015, we collected adult lizards (14♂:14♀) from a population of *S. tristichus* in Navajo County, Arizona (elevation = 1930 m). These lizards were transported to Arizona State University, where they were housed individually in plastic terraria (30 × 20 × 20 cm). The terraria were kept in an incubator (Percival DR-36VL; Percival Scientific, Perry, IA, USA) that maintained a diel cycle of 33 °C for 8 h of photophase and 20 °C for 14 h of scotophase. Lizards were offered crickets (*Acheta domesticus*) and water daily. The experiments were conducted approximately 3 months after lizards were collected in the field to provide sufficient time for females to lay eggs and regain weight. Snout–vent lengths ranged from 55 to 66 mm in males and from 48 to 69 mm in females.

We measured the voluntary thermal maximum of each lizard at 3 normobaric concentrations of oxygen: 13%, 21% and 29%. Measurements were made at one oxygen concentration per day, using a random order of concentrations for each lizard. Each lizard was placed in a cylindrical aluminum chamber (400 mL), warmed by a 60-W incandescent bulb (Fig. 1). The chamber resided in a Styrofoam box (22 × 22 × 32 cm), which, in turn, resided in a transparent plastic container (67 × 41 × 31 cm). Prior to placing a lizard in the chamber, the oxygen concentration of the box was set with an electronic oxygen controller (ROXY-1, Sable Systems International, Las Vegas, NV, USA). Throughout the experiment, the ROXY-1 continuously monitored oxygen concentration through a sensor that was placed inside the plastic box, just outside the Styrofoam container. The ROXY-1 automatically added nitrogen or oxygen as needed to maintain the desired concentration of oxygen (±0.5%).

During a trial, a lizard was placed in the chamber and heated 0.7 °C·min^{-1} (range = 0.2–1.8 °C·min^{-1}) from an

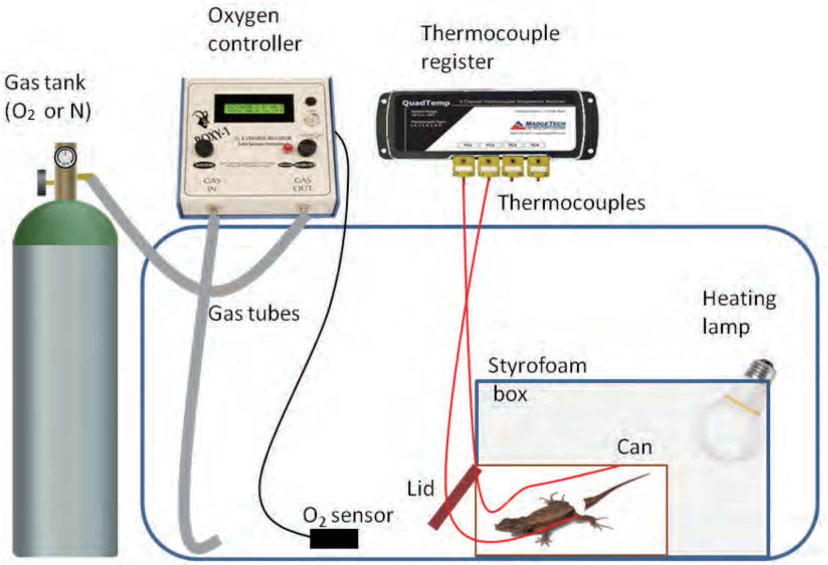

Figure 1 The voluntary thermal maximum of lizards was measured in a dark, metallic chamber, warmed from the outside with a fluorescent bulb. The lizard could escape the heat by exiting the chamber through a partially opened lid. Oxygen concentration was controlled by a Roxy-1 O_2 Regulator connected an oxygen sensor in front of the chamber. The temperature of the chamber wall and the temperature of the lizard were recorded with thermocouples connected to a Madgetech Temperature Recorder.

initial temperature of 33 °C, the preferred body temperature of the species (Buckley *et al*. 2015). Temperatures were recorded every 5 s during the trial. Temperature inside the aluminum chamber was monitored by a thermocouple attached to the inner surface. A plastic flap at one end of the chamber enabled the animal to exit and experience room temperature (approximately 25 °C). A thermocouple wire was also taped to the groin of the lizard, enabling us to record skin temperature while permitting lizards to move. Using this method previously, we found that differences between the temperature of the cloaca and the temperature of the groin were smaller than the error of the thermocouples (Camacho *et al.*, in review).

While warming, the lizard could explore the chamber and exit through the plastic flap if desired. We defined the voluntary thermal maximum as the body temperature at which a lizard's head or limb first protruded from the chamber. We recorded the temperature and time when this event occurred, before returning lizards to their terrarium. The mean rate of heating was calculated from the time taken to heat from the initial temperature to the voluntary thermal maximum.

To quantify sources of variation in the voluntary thermal maximum, we fit general linear mixed models using the *nlme* library of the R Statistical Package (R Core Team 2014). Oxygen concentration and sex were fixed factors, heating rate was a covariate, and the identity of the lizard was a random factor. We omitted snout–vent length because this variable was uncorrelated with the voluntary thermal maximum ($r^2 = 0.01$); however, any effect of body size was absorbed in the random effect of the individual. Following Burnham and Anderson (2002), we used multimodel averaging to estimate the most likely values of means. First, we used the *MuMIn* library (Bartoń 2013) to fit all possible models to the data. Then, we calculated the Akaike information criterion and Akaike weight of each model, the latter variable being the probability that the model best describes the data (Table 1). Finally, we calculated the weighted average of each parameter including estimates from all models. The resulting values of parameters were used to calculate the most likely mean for each combination of factors.

RESULTS

Oxygen supply had no discernable effect on the voluntary maximum temperature of lizards. The most likely models excluded an effect of oxygen concentration (Table 1). By summing the Akaike weights for all mod-

Table 1 Factors affecting the voluntary thermal maximum of 28 adult *S. tristichus* lizards lizards (14♂:14♀), according to a model averaging procedure. The most likely model of the voluntary thermal maximum included an effect of heating rate but no effect of oxygen concentration.

Terms in the model	*K*	log *L*	AIC	ΔAIC	*w*
Heating rate	4	−132.1	272.7	0	0.31
Heating rate + sex	5	−131.6	274.0	1.33	0.16
Null	3	−134.1	274.6	1.92	0.12
Heating rate + sex + (heating rate · sex)	6	−130.8	274.7	2.02	0.11
Sex	4	−133.5	275.5	2.84	0.07
Oxygen + heating rate	6	−131.2	275.5	2.85	0.07
Oxygen + heating rate + sex + (heating rate · sex)	8	−129.4	276.8	4.10	0.04
Oxygen + heating rate + sex	7	−130.8	277.0	4.37	0.03
Oxygen	5	−133.4	277.6	4.96	0.03
Oxygen + sex	6	−132.8	278.7	6.04	0.02
Oxygen + heating rate + (oxygen · heating rate)	8	−130.6	279.1	6.46	0.01
Oxygen + heating rate + sex + (oxygen · heating rate)	9	−129.8	280.0	7.39	0.01

Models are ranked according to their Akaike information criterion (AIC), which depends on the log likelihood (log *L*) and the number of parameters (*K*). For each model, we provide the Akaike weight (*w*), which equals the probability that the model describes the data better than the other models. All models contained an intercept and a random effect of individuals.

els with an effect of oxygen, we estimated the likelihood that oxygen was an important factor to be only 23%. Although some individuals had higher or lower voluntary thermal maxima when exposed to lower concentrations of oxygen, the effect of oxygen concentration estimated by multi-model averaging was close to zero (Fig. 2). The 2 most likely models included an effect of heating rate, although this effect accounted for a small proportion of the variation (Fig. 3). On average, faster heating decreased the voluntary thermal maximum by 1 °C when comparing values obtained from heating lizards at the slowest and fastest rates (see Fig. 3). The second-best model also included an effect of sex, but this effect was even weaker than the effect of heating rate; the mean voluntary thermal maximum of males exceeded that of females by only 0.2 °C (see Fig. 3).

DISCUSSION

Although some species of lizards exposed to severe hypoxia prefer lower temperatures or thermoregulate less precisely (Hicks & Wood 1985; Branco *et al.* 1993; Bicego-Nahas *et al.* 2001; Cadena & Tattersall 2010; He *et al.* 2013; Li *et al.* 2016), our results make us question the ecological relevance of these results. No active lizard experiences an atmospheric concentration of oxygen below 10%, or its partial-pressure equivalent, either now or in recent history (Berner *et al.* 2007). Even if climate warming pushed the range of a species to the top of a mountain, a lizard would experience a partial pressure of oxygen that equates to a concentration of 13% oxygen at sea level. Neither this realistic level of hypoxia nor the hyperoxic treatment had a discernable effect on a lizard's voluntary thermal maximum. Lizards remained in the chamber until body temperature reached an average of 40.8 °C (SD = 1.2 °C): extremely close to the critical thermal maximum of 41.1 °C (SD = 1.2 °C) reported by Buckley and colleagues (2015). Thus, lizards likely experienced thermal stress for reasons other than a shortage of oxygen in the environment.

Because metabolism depends on the capacity to transport oxygen from the lungs to other tissues, we should consider the carrying capacity of blood in a hypoxic atmosphere. Two factors determine the amount of oxygen that a reptile can supply to its tissues as body temperature rises: the amount of hemoglobin and its degree of saturation. Because reptiles have a heart with partially divided ventricles, they can shunt deoxygenated blood from the systemic circuit to the pulmonary circuit as tissues demand more oxygen (Wang & Hicks 2002). Such shunting would oxygenate more hemoglobin and compensate for the greater oxygen demand during heating (Hicks & Wang 1996). In addition, both temperature and PO_2 determine saturation of hemoglobin. At temperatures near the voluntary thermal maximum, hemoglobin would be right-shifted, decreasing its affinity for oxygen and increasing the chance of being unsaturated at normoxia (Wood 1991, 1995; Burmester & Hankeln 2014). In theory, a hypoxic atmosphere amplifies the loss of affinity at high temperatures (Wood *et al.* 1987). How-

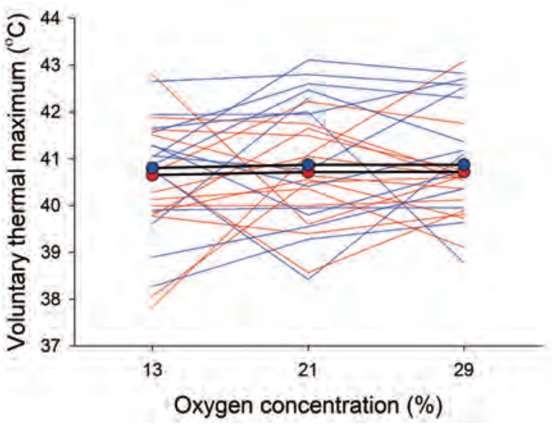

Figure 2 Oxygen concentration had no effect on the mean voluntary thermal maximum of lizards. Blue and red symbols denote model-averaged means for males and females, respectively. Lines show raw data for individuals.

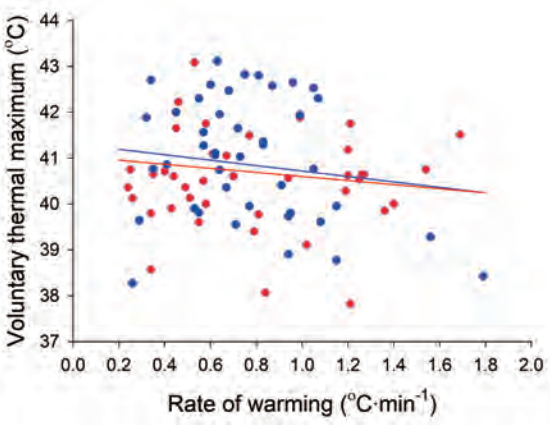

Figure 3 Lizards that warmed faster had a lower voluntary thermal maximum. Blue and red lines denote model-averaged relationships for males and females, respectively. Points show raw data for individuals.

ever, the lack of an effect of hypoxia on the voluntary thermal maximum suggests that lizards did not reach the saturation limit during warming. In which case, greater cardiac output and pulmonary ventilation might have compensated for the drop in atmospheric oxygen (Boyer 1966; Dawson & Templeton 1966; Glass & Wood 1983).

The demand for oxygen also plays a role in behavioral hypothermia. We studied the effect of hypoxia on resting animals; however, active animals face a greater demand for oxygen that makes them more susceptible to hypoxia. Activity may exacerbate the effect of hypoxia on preferred body temperature because of the lactate that accumulates during anaerobic metabolism. Hence, studies of active animals could reveal behavioral hypothermia even at moderate levels of hypoxia. This hypothesis accords with experimental studies of toads conducted by Pörtner and his colleagues. During exercise, lactate concentration in plasma increases before an animal reaches its critical PO_2 (Portner *et al.* 1991). Eventually, this lactate must be converted to pyruvate, which occurs faster when muscles have oxygen. Therefore, animals needing to sustain locomotor activity or repay oxygen debt should be more sensitive to hypoxia than resting animals. In support of this model, Pörtner and colleagues (1994) were able to stimulate behavioral hypothermia by infusing lactate in the blood of toads. Furthermore, infusions of lactate caused toads to consume oxygen faster (Pinz & Portner 2003), presumably to repay a perceived oxygen debt. Given these results, we believe future work should focus on behavioral hypothermia in active animals exposed to hypoxia.

Although the voluntary thermal maximum of adult lizards appears insensitive to hypoxia, other life stages may be affected more strongly by this change in oxygen (Flewelling & Parker 2015). For example, embryos of *S. tristichus* suffered an exponential loss of thermal tolerance as the atmospheric concentration of oxygen dropped from 21% to 12% (Smith *et al.* 2015). This greater susceptibility to hypoxia could stem from two factors. First, the cardiovascular and respiratory systems of embryos are at a less developed stage than that of adult lizards. Second, the delivery of oxygen depends on diffusion across the egg shell, which would be compounded in the mother's uterus by the lower solubility of oxygen in plasma than in air (Andrews 2002). As another case study, pregnant females have greater metabolic demands (Angilletta & Sears 2000), potentially making them more susceptible to hyperthermia. Although females do not necessarily choose lower temperatures when carrying eggs (Angilletta *et al.* 2000), they sometimes increase thermoregulatory precision to avoid extreme heat (Mathies & Andrews 1997). Nevertheless, we cannot currently evaluate this hypothesis because experiments comparing the thermoregulatory performance of pregnant females to that of non-pregnant females have been conducted only under normoxia (Beuchat & Ellner 1987; Mathies & Andrews 1997; Angilletta *et al.* 2000).

Because montane species will likely shift to higher elevations as climates warm, biologists should continue to question whether oxygen supply sets the thermal tolerance of terrestrial animals and limits their geographic range. Although heat tolerances of aquatic animals are often limited by oxygen supply (Verberk *et al.* 2016), air breathing animals likely overheat for reasons other than an insufficient supply of oxygen (Klok *et al.* 2004; McCue & Santos 2013; Fobian *et al.* 2014). However, this conclusion holds only for resting animals, which have far lower demands for oxygen than active animals. Once an animal becomes active, its performance at high temperatures may depend on its ability to acquire oxygen (Teague *et al.* 2017). Moreover, adults of many terrestrial species begin life as embryos or larvae developing in an aqueous environment, which could limit oxygen consumption during development. A population can decline if the additive or interactive effects of hypoxia and hyperthermia reduce growth or survival of individuals early in the life cycle. Although moderate hypoxia failed to affect the behavior of adult lizards in our experiment, it does reduce the heat tolerance of embryonic lizards (Smith *et al.* 2015). Thus, we should ask whether this interaction between PO_2 and temperature will prevent lizards from dispersing to higher elevations. Not only might abiotic factors such as PO_2 interact with temperature, but so might biotic factors such as the quality and quantity of competitors or mutualists. For example, the heat tolerance of some animals depends on their community of endosymbionts (Mandrioli & Manicardi 2013; Schwab *et al.* 2016; Ziegler *et al.* 2017). The strength of this interaction likely changes throughout the life cycle, just as the interaction between temperature and PO_2 does. Clearly, biologists should consider the significance of these interactions by modeling multiple dimensions of the niche.

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REFERENCES

Andrews RM (2002). Low oxygen: A constraint on the evolution of viviparity in reptiles. *Physiological and Biochemical Zoology* **75**, 145−54.

Angert AL, LaDeau SL, Ostfeld RS (2013). Climate change and species interactions: Ways forward. *Annals of the New York Academy of Sciences* **1297**, 1−7.

Angilletta MJ, Winters RS, Dunham AE (2000). Thermal effects on the energetics of lizard embryos: Implications for hatchling phenotypes. *Ecology* **81**, 2957−68.

Bartoń K (2013). MuMIn: Multi-model inference, R package version 1.9.13.

Berner RA, VandenBrooks JM, Ward PD (2007). Evolution − Oxygen and evolution. *Science* **316**, 557−8.

Beuchat CA, Ellner S (1987). A quantitative test of life-history theory: Thermoregulation by a viviparous lizard. *Ecological Monographs* **57**, 45−60.

Bicego-Nahas KC, Gargaglioni LH, Branco LGS (2001). Seasonal changes in the preferred body temperature, cardiovascular, and respiratory responses to hypoxia in the toad, *Bufo paracnemis*. *Journal of Experimental Zoology* **289**, 359−65.

Boyer DR (1966). Comparative effects of hypoxia on respiratory and cardiac function in reptiles. *Physiological Zoology* **39**, 307−16.

Branco LGS, Malvin GM (1996). Thermoregulatory effects of cyanide and azide in the toad, Bufo marinus. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* **39**, R169−73.

Branco LG, Portner HO, Wood SC (1993). Interaction between temperature and hypoxia in the alligator. *American Journal of Physiology − Regulatory, Integrative and Comparative Physiology* **265**, R1339.

Buckley LB (2010). The range implications of lizard traits in changing environments. *Global Ecology and Biogeography* **19**, 452−64.

Buckley LB, Kingsolver JG (2012). Functional and phylogenetic approaches to forecasting species' responses to climate change. *Annual Review of Ecology, Evolution, and Systematics* **43**, 205–26.

Buckley LB, Urban MC, Angilletta MJ, Crozier LG, Rissler LJ, Sears MW (2010). Can mechanism inform species' distribution models? *Ecology Letters* **13**, 1041–54.

Buckley LB, Ehrenberger JC, Angilletta MJ (2015). Thermoregulatory behavior limits local adaptation of thermal niches and confers sensitivity to climate change. *Functional Ecology* **29**, 1038–47.

Burmester T, Hankeln T (2014). Function and evolution of vertebrate globins. *Acta Physiologica* **211**, 501–14.

Burnham KP, Anderson DR (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer, New York.

Cadena V, Tattersall GJ (2010). Decreased precision contributes to the hypoxic thermoregulatory responses in lizards. *Journal of Experimental Biology* **212**, 137–44.

Camacho A, Rodrigues MT, Navas C (2015). Extreme operative temperatures are better descriptors of the thermal environment than mean temperatures. *Journal of Thermal Biology* **49**, 106–11.

Chapman DS, Scalone R, Stefanic E, Bullock JM (2017). Mechanistic species distribution modeling reveals a niche shift during invasion. *Ecology* **98**, 1671–80.

Cope ED (1875). In: Yarrow HC. Report upon the collections of batrachians and reptiles made in portions of Nevada, Utah, California, Colorado, New Mexico, and Arizona, during the years 1871, 1872, 1873, and 1874, Chapter IV, L, pp. 509–84. In: Report upon Geography. Government Printing office, Washington D.C.

Dawson WR, Templeton JR (1966). Physiological responses to temperature in the alligator lizard, *Gerrhonotus multicarinatus*. *Ecology* **47**, 759–65.

de Velasco JB, Tattersall GJ (2008). The influence of hypoxia on the thermal sensitivity of skin colouration in the bearded dragon, *Pogona vitticeps*. *Journal of Comparative Physiology B* **178**, 867–75.

Dillon ME, Frazier MR, Dudley R (2006). Into thin air: Physiology and evolution of alpine insects. *Integrative and Comparative Biology* **46**, 49–61.

Flewelling S, Parker SL (2015). Effects of temperature and oxygen on growth and differentiation of embryos of the ground skink, Scincella lateralis. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* **323**, 445–55.

Fobian D, Overgaard J, Wang T (2014). Oxygen transport is not compromised at high temperature in pythons. *Journal of Experimental Biology* **217**, 3958–61.

Glass ML, Wood SC (1983). Gas exchange and control of breathing in reptiles. *Physiological Reviews* **63**, 232.

He J, Xiu M, Tang X *et al.* (2013). Thermoregulatory and metabolic responses to hypoxia in the oviparous lizard, Phrynocephalus przewalskii. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **165**, 207–13.

Hicks JW, Wang T (1996). Functional role of cardiac shunts in reptiles. *Journal of Experimental Zoology* **275**, 204–16.

Hicks JW, Wang T (2004). Hypometabolism in reptiles: Behavioural and physiological mechanisms that reduce aerobic demands. *Respiratory Physiology & Neurobiology* **141**, 261–71.

Hicks JW, Wood SC (1985). Temperature regulation in lizards: Effects of hypoxia. *American Journal of Physiology* **248**, R595–600.

Kearney M, Phillips BL, Tracy CR, Christian KA, Betts G, Porter WP (2008). Modelling species distributions without using species distributions: The cane toad in Australia under current and future climates. *Ecography* **31**, 423–34.

Kearney M, Porter W (2009). Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges. *Ecology Letters* **12**, 334–50.

Kearney M, Predavec M (2000). Do nocturnal ectotherms thermoregulate? A study of the temperate gecko Christinus marmoratus. *Ecology* **81**, 2984–96.

Kearney M, Simpson SJ, Raubenheimer D, Helmuth B (2010a). Modelling the ecological niche from functional traits. *Philosophical Transactions of the Royal Society B* **365**, 3469–83.

Kearney MR, Briscoe NJ, Karoly DJ, Porter WP, Norgate M, Sunnucks P (2010b). Early emergence in a butterfly causally linked to anthropogenic warming. *Biology Letters* **6**, 674–7.

Klok CJ, Sinclair BJ, Chown SL (2004). Upper thermal tolerance and oxygen limitation in terrestrial arthropods. *Journal of Experimental Biology* **207**, 2361–70.

Levy O, Buckley LB, Keitt TH, Angilletta MJ (2016. Ontogeny constrains phenology: Opportunities for activity and reproduction interact to dictate potential phenologies in a changing climate. *Ecology Letters* **19**, 620–8.

Levy O, Buckley LB, Keitt TH *et al.* (2015). Resolving the life cycle alters expected impacts of climate change. *Proceeding of the Royal Society B* **282**, 20150837.

Li WX, Liang SW, Wang HH *et al.* (2016). The effects of chronic hypoxia on thermoregulation and metabolism in *Phrynocephalus vlangalii*. Asian Herpetolgical Research **7**, 103–11.

Mandrioli M, Manicardi GC (2013). Evolving aphids: One genome-one organism insects or holobionts? *Invertebrate Survival Journal* **10**, 1–6.

Martin TL, Huey RB (2008). Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. *American Naturalist* **171**, E102–18.

Mathies T, Andrews RM (1997). Influence of pregnancy on the thermal biology of the lizard, Sceloporus jarrovi: Why do pregnant females exhibit low body temperatures? *Functional Ecology* **11**, 498–507.

McCue MD, Santos RD (2013). Upper thermal limits of insects are not the result of insufficient oxygen delivery. *Physiological and Biochemical Zoology* **86**, 257–65.

Parmesan C, Yohe G (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37–42.

Petric M, Lalic B, Ducheyne E, Djurdjevic V, Petric D (2017). Modelling the regional impact of climate change on the suitability of the establishment of the Asian tiger mosquito (*Aedes albopictus*) in Serbia. *Climate Change* **142**, 361–74.

Pinz I, Portner HO (2003). Metabolic costs induced by lactate in the toad *Bufo marinus*: new mechanism behind oxygen debt? *Journal of Applied Physiology* **94**, 1177–85.

Pörtner HO (2001). Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**, 137–46.

Pörtner HO (2002a). Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology A* **132**, 739–61.

Pörtner HO (2002b. Physiological basis of temperature-dependent biogeography: Trade-offs in muscle design and performance in polar ectotherms. *Journal of Experimental Biology* **205**, 2217–30.

Pörtner HO, Branco LGS, Malvin GM, Wood SC (1994). A new function for lactate in the toad *Bufo marinus*. *Journal of Applied Physiology* **76**, 2405–10.

Portner HO, Maclatchy LM, Toews DP (1991). Metabolic responses of the toad Bufo marinus to environmental hypoxia: an analysis of the critical PO_2. *Physiological Zoology* **64**, 836–49.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from URL: http://www.R-project.org/.

Schwab DB, Riggs HE, Newton ILG, Moczek AP (2016). Developmental and ecological benefits of the maternally transmitted microbiota in a dung beetle. *American Naturalist* **188**, 679–92.

Sinclair BJ, Marshall KE, Sewell MA *et al.* (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters* **19**, 1372–85.

Smith C, Telemeco RS, Angilletta MJ, VandenBrooks JM (2015). Oxygen supply limits the heat tolerance of lizard embryos. *Biology Letters* **11**, 20150113.

Steiner AA, Branco LGS (2001). Nitric oxide in the regulation of body temperature and fever. *Journal of Thermal Biology* **26**, 325–30.

Steiner AA, Branco LGS (2002). Hypoxia-induced anapyrexia: Implications and putative mediators. *Annual Review of Physiology* **64**, 263–88.

Tamaki Y, Nakayama T (1987). Effects of air constituents on thermosensitivies of preoptic neurons: hypoxia versus hypercapnia. *Pflugers Archiv-European Journal of Physiology* **409**, 1–6.

Teague C, Youngblood J, Ragan K, Angilletta MJ, VandenBrooks JM (2017). A positive genetic correlation between hypoxia tolerance and heat tolerance supports a controversial theory of heat stress. *Biology Letters* **13**, 20170309 .

Vasseur DA, DeLong JP, Gilbert B *et al.* (2014). Increased temperature variation poses a greater risk to species than climate warming. *Proceedings of the Royal Society B* **281,** 20132612.

Verberk W, Overgaard J, Ern R *et al.* (2016). Does oxygen limit thermal tolerance in arthropods? A critical review of current evidence. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **192**, 64–78.

Wang T, Hicks JW (2002). An integrative model to predict maximum O2 uptake in animals with central vascular shunts. *Zoology* **105**, 45–53.

Wood SC (1991). Interactions between hypoxia and hypothermia. *Annual Review of Physiology* **53**, 71–85.

Wood SC (1995). Oxygen as a modulator of body-temperature. Brazilian Journal of Medical and Biological Research **28**, 1249–56.

Wood SC, Gonzales R (1996). Hypothermia in hypoxic animals: Mechanisms, mediators, and functional significance. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology* **113**, 37–43.

Wood SC, James WH, Dupré RK (1987). Hypoxic reptiles: Blood gases, body temperature and control of breathing. *American Zoologist* **27**, 21–9.

Ziegler M, Seneca FO, Yum LK, Palumbi SR, Voolstra CR (2017). Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nature Communications* **8**, 14213.

Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM (2009). *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York.

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