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INVITED PERSPECTIVES

High Temperature, Oxygen, and Performance: Insights from Reptiles and Amphibians

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Synopsis Much recent theoretical and empirical work has sought to describe the physiological mechanisms underlying thermal tolerance in animals. Leading hypotheses can be broadly divided into two categories that primarily differ in organizational scale: 1) high temperature directly reduces the function of subcellular machinery, such as enzymes and cell membranes, or 2) high temperature disrupts system-level interactions, such as mismatches in the supply and demand of oxygen, prior to having any direct negative effect on the subcellular machinery. Nonetheless, a general framework describing the contexts under which either subcellular component or organ system failure limits organisms at high temperatures remains elusive. With this commentary, we leverage decades of research on the physiology of ectothermic tetrapods (amphibians and non-avian reptiles) to address these hypotheses. Available data suggest both mechanisms are important. Thus, we expand previous work and propose the Hierarchical Mechanisms of Thermal Limitation (HMTL) hypothesis, which explains how subcellular and organ system failures interact to limit performance and set tolerance limits at high temperatures. We further integrate this framework with the thermal performance curve paradigm commonly used to predict the effects of thermal environments on performance and fitness. The HMTL framework appears to successfully explain diverse observations in reptiles and amphibians and makes numerous predictions that remain untested. We hope that this framework spurs further research in diverse taxa and facilitates mechanistic forecasts of biological responses to climate change.

Introduction

For most animals, the proximate mechanisms that underlie reduced performance and eventual death at high temperatures, and how such mechanisms might change with ontogeny and context, are uncertain (reviewed in Angilletta 2009; Clark et al. 2013; Pörtner et al. 2017). Such a mechanistic understanding would greatly enhance our ability to predict the effects of realistic high-temperature exposures on individuals or populations, which are difficult to forecast with traditional methods because responses can be highly variable (e.g., Gunderson and Stillman 2015; Seebacher et al. 2015; Kingsolver and Woods 2016; Sheldon and Dillon 2016; Williams et al. 2016). Even so, much progress has been made toward developing mechanistic models that use knowledge of individual physiology to predict the effects of thermal environments on populations, largely inspired by growing concern over the impacts of global climate change (Buckley 2008; Kearney and Porter 2009; Huey et al. 2012; Kearney 2012; Levy et al. 2015; Malishev et al. 2018). Commonly, such models employ empirically-derived thermal performance curves (TPC, Huey and Stevenson 1979, all terms and abbreviations are defined in Box 1) to predict the effects of thermal environments on organisms and populations (e.g. Colwell et al. 2008; Deutsch et al. 2008; Vasseur et al. 2014; Buckley and Huey 2016; Dillon et al. 2016). TPCs describe how performance varies with temperature—typically performance increases with temperature above a critical minimum (CT_{MIN}) until an optimum is reached (T_{OPT}), then rapidly drops to zero at the critical maximum (CT_{MAX}, see Fig. 1 for examples). Pejus (getting worse) temperatures above and below

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Box 1 List of terms and abbreviations

ATP	Adenosine triphosphate
active-aerobic T_{CRIT}	Critical temperature where aerobic respiration is maximized for active individuals (i.e., $\dot{V}_{O_2MAX}$ is maximized, °C)
resting-aerobic T_{CRIT}	Critical temperature where aerobic respiration would be maximized for resting individuals, assuming they survive to such high temperatures (i.e., $\dot{V}_{O_2REST}$ is maximized, °C)
subcellular T_{CRIT}	Critical temperature for subcellular function (°C)
CT_{MAX}	Critical thermal maximum (°C)
HMTL	Hierarchical Mechanisms of Thermal Limitation hypothesis
HSP	Heat shock protein
MPMO	Multiple Performances – Multiple Optima hypothesis (sensu Clark et al. 2013)
OCLTT	Oxygen- and Capacity-Limited Thermal Tolerance hypothesis (sensu Pörtner 2002 and Pörtner et al. 2017)
T_{GAPE}	Gaping temperature: Temperature at which animals gape to promote evaporative cooling (°C)
T_{LETHAL}	Lethal temperature: Temperature at which an organism dies under acute exposure (°C)
T_{OPT}	Optimal temperature for performance (°C)
T_{PANT}	Panting temperature: Temperature at which animals pant to promote evaporative cooling (°C)
T_{PEJUS}	Pejus (i.e. getting worse) temperature (°C, sensu Frederich and Pörtner 2000)
TPC	Thermal performance curve (sensu Huey and Stevenson 1979)
PBT	Preferred body temperature (°C)
$P_{CT_{MAX}}$	Oxygen partial pressure below which CT_{MAX} is reduced (kPa) (sensu Ern et al. 2016)
P_{O_2}	Partial pressure of oxygen (kPa)
P_{CO_2}	Partial pressure of carbon dioxide (kPa)
$\dot{V}_{O_2}$	Oxygen consumption rate (generally mL O_2 min^{-1})

T_{OPT} are described by breakpoints in physiological function (e.g., ventilation rate, heart rate, P_{O_2}) indicative of rapid declines in whole-organism performance (T_{PEJUS}, Frederich and Pörtner 2000; Pörtner 2002; Pörtner et al. 2017). Typically, TPCs are estimated under controlled laboratory conditions for a single trait and time, but TPC shape can vary with season, ontogeny, trait, and prior experience in ways that are difficult to predict (Rezende et al. 2014; Telemeco 2014; Kingsolver and Woods 2016; Williams et al. 2016). Thus, using empirically-derived TPCs to predict the effects of natural environments on performance is problematic because it requires extrapolating from the traits or environments originally used to estimate TPCs, thereby ignoring probable context-dependency. A mechanistic understanding of the processes that underlie thermal performance is needed to predict the effects of variable or novel environments on TPC shape, which will greatly improve models relying on TPCs to predict population responses. In particular, knowledge of the mechanisms that result in loss of function at high temperatures is needed to predict the rate at which performance will drop in response to thermal challenge, the capacity for animals to recover from sublethal thermal exposure, and the capacity for thermal tolerance to change via plasticity or evolution (Helmuth et al. 2005; Buckley and Huey 2016; Williams et al. 2016).

Potential mechanisms explaining why animals lose function at high temperatures can be divided into two major categories differing in the level of organization first affected. At lower levels of organization, subcellular components could be critically impaired when animals are exposed to temperatures above the optimum. Subcellular impairment results from either denaturation of key molecules, such as proteins and cell membranes, or reduced efficiency of these molecules to perform their biological functions (reviewed in Hochachka and Somero 2002; Angilletta 2009; Schulte 2015). Impairment of subcellular components would result in the breakdown of higher levels of organization and lead to rapid performance loss. Alternatively, higher-levels of organization, such as organ systems, could be impaired at temperatures below those that directly affect the performance of their subcellular components if high temperatures disrupt subcellular interactions or pathways necessary for organ system function. A recent mechanistic model explaining organ-system impairment at high temperatures is the oxygen and capacity limited thermal tolerance (OCLTT) hypothesis, which proposes that oxygen demand for aerobic metabolism at high temperatures outpaces the ability of the

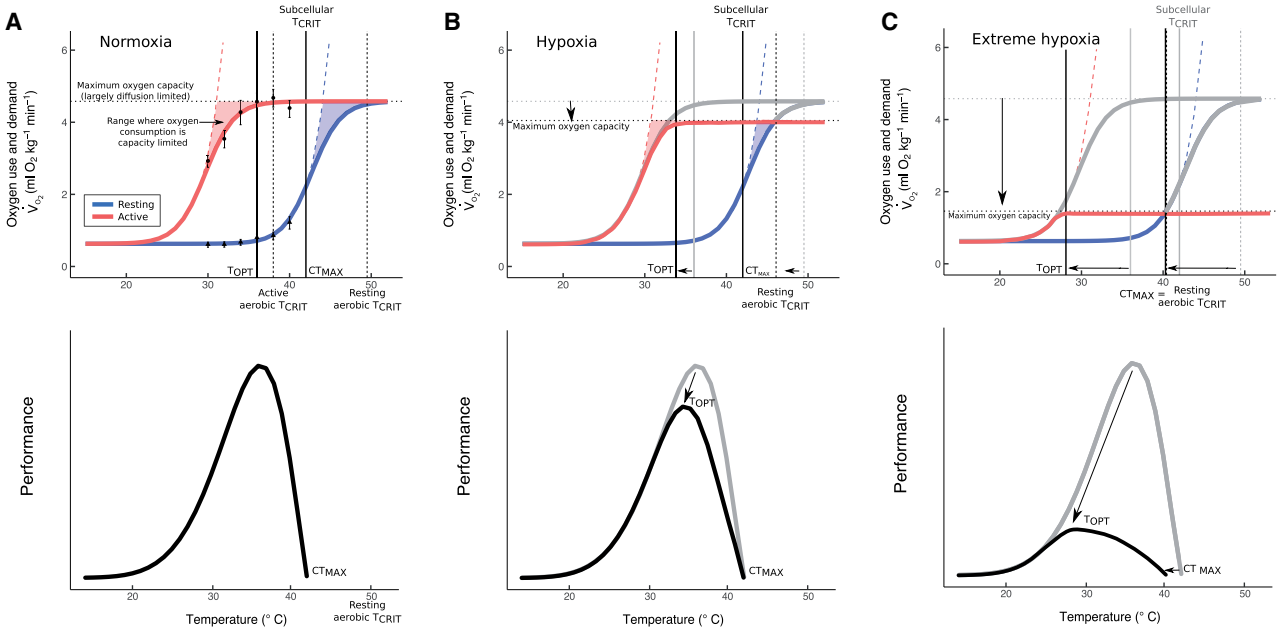

Fig. 1 Schematic of the HMTL framework illustrating proposed relationships between body temperature, oxygen environment, metabolism, thermal limits, and performance. The top row of plots displays resting and active metabolic rates as a function of temperature within thermal and physiological limits, and the bottom row displays predicted thermal performance curves. Panel (**A**) displays these relationships in normoxic environments. Data are means ± s.e.m. for the snake, *P. regius*, from Fobian et al. (2014). These data were used to fit curves for resting and active metabolic rate, which were then used to estimate T_{OPT} (maximal aerobic scope), resting-aerobic T_{CRIT}, active-aerobic T_{CRIT}, and thermal performance curve shape. The critical thermal maximum is 42°C (Fobian et al. 2014). Panel (**B**) Moderate hypoxia (10–20 kPa) is predicted to have no effect on CT_{MAX} or resting metabolism because resting aerobic T_{CRIT} is above subcellular T_{CRIT}. Even so, active-aerobic T_{CRIT} will be reduced thereby lowering aerobic scope, maximal performance, and T_{OPT}. Panel (**C**) Exposure to extreme hypoxia (< 10 kPa) will reduce CT_{MAX} because resting-aerobic T_{CRIT} drops below subcellular T_{CRIT}. Active-aerobic T_{CRIT} will be strongly affected by such hypoxic environments as well, with large reductions in aerobic scope, maximal performance, and T_{OPT}. In Panels B) and C), gray lines illustrate values predicted for normoxia and are included to aid interpretation of changes predicted to result from reduced environmental oxygen availability. Similarly, arrows in Panels B) and C) indicate predicted displacement of maximum oxygen capacity, resting-aerobic T_{CRIT}, T_{OPT}, and CT_{MAX} values given the oxygen environment. Subcellular T_{CRIT} is not predicted to be affected by oxygen environment and therefore is never displaced.

cardiovascular and respiratory systems to provide sufficient amounts of oxygen to tissues (Pörtner 2002; Pörtner and Knust 2007; Verberk et al. 2016; Pörtner et al. 2017). Under this hypothesis, oxygen diffusion and transport capacity limit metabolic rates such that resting rates converge with potential maximum rates at high temperatures thereby reducing aerobic scope to zero (i.e., zero aerobic power budget sensu Pörtner et al. 2017). This mismatch in supply and demand will first reduce aerobic performance at T_{PEJUS} but eventually result in basal oxygen demands not being met and thus collapse of organismal systems at CT_{MAX}. Proponents of OCLTT argue that this mechanism integrates across levels of organization from systemic to molecular mechanisms and can explain diverse evolutionary and ecological phenomena (Pörtner 2002; Pörtner et al. 2017). However, the temporal window, activity range, magnitude, and biological relevancy of reduced aerobic scope at high temperature are debated (Clark et al. 2013; Gräns et al. 2014; Jutfelt et al. 2014; Pörtner 2014; Verberk et al. 2016; Jutfelt et al. 2018). Identifying the level of organization first impaired by high temperature is necessary to understand how temperature affects whole-organism function.

Recent work exploring the mechanisms underlying thermal tolerance in ectotherms focuses on aquatic taxa (e.g., Frederich and Pörtner 2000; Lucassen et al. 2006; Verberk et al. 2013; Gräns et al. 2014; Ern et al. 2016; Pörtner and Gutt 2016; Verberk et al. 2018) and terrestrial arthropods, particularly insects (reviewed in McCue and De Los Santos 2013; Verberk et al. 2016). These observations suggest that subcellular-level mechanisms are more important determinants of thermal tolerance than oxygen capacity limitations in terrestrial taxa, perhaps because oxygen is more abundant in air than in water. However, recent work includes few studies of terrestrial vertebrate ectotherms: non-avian reptiles (hereafter, reptiles) and amphibians. Reptiles and amphibians display varied ecology, life-history, and thermal tolerance (Vitt and Caldwell 2009; Sunday

et al. 2011) and are important for a broad understanding of thermal tolerance. Additionally, these animals frequently contend with challenging temperatures, with some species inhabiting the hottest terrestrial environments (Cowles and Bogert 1944; Vitt and Caldwell 2009; Sunday et al. 2014). Moreover, both the thermal and oxygen environment can vary throughout ontogeny (e.g., aquatic to terrestrial transition in amphibians), and modes of respiration can vary (e.g., cutaneous respiration in many amphibians and some reptiles, such as turtles; Hutchison et al. 1968; Glass et al. 1981; Wang 2011). Because of these traits, reptiles and amphibians have been important models for thermal physiology and ecology for the last century (e.g., Cowles and Bogert 1944; Snyder and Weathers 1975; Huey and Stevenson 1979; Huey 1982; Huey and Berrigan 2001; Vitt and Caldwell 2009; Kearney 2012). This historic work can be leveraged to address modern ideas, such as the OCLTT hypothesis. Furthermore, these taxa are at risk of high-temperature induced extinction resulting from global climate change (Thomas et al. 2004; Huey et al. 2010; Rohr and Raffel 2010; Sinervo et al. 2010; Kearney 2013). Here, we apply the rich history of physiological studies in reptiles and amphibians to modern hypotheses for mechanisms underlying thermal performance and tolerance. This will complete taxonomic coverage of recent reviews, and, more importantly, further understanding of how organismal performance and survival are restricted by high temperatures.

In this commentary, we begin by synthesizing evidence for subcellular- and organ-system-level failures underlying high temperature tolerance and performance in reptiles and amphibians. Because neither framework satisfactorily explains observed patterns individually, we next build upon work in other taxa to present an integrative framework combining both subcellular and organ-system sensitivity to high temperatures and the thermal performance curve paradigm. Using data for reptiles and amphibians, we illustrate how this combined framework appears to predict a broad array of physiological and behavioral observations. We conclude by identifying future research directions to test this framework and discuss how it might be applied to other systems to predict consequences of future high-temperature exposure for organisms and populations.

Evidence for mechanisms underlying thermal tolerance and performance in reptiles and amphibians

Subcellular mechanisms

High temperatures compromise the structure and function of subcellular components, such as enzymes and cell membranes (Fields 2001; Hochachka and Somero 2002) but such damage will only underlie organismal thermal tolerance if it occurs at temperatures below those affecting function at higher levels of organization, such as organ systems (Pörtner 2002). The complexities of enzymatic interactions make extending observations for individual reactions to the whole cell or organism potentially problematic (Schulte 2015). Nonetheless, the observation that subcellular components are frequently stable to temperatures above the critical and lethal limits of animals (hereafter, CT_{MAX} and T_{LETHAL}), commonly maintaining function to temperatures >50°C and capable of evolving stability to 120°C (Fields 2001), was an important motivation for the development of hypotheses such as OCLTT (Pörtner 2001; 2002; Pörtner et al. 2017). For example, CT_{MAX} in reptiles and amphibians generally ranges from mid-30s to low 40s °C (Brattstrom 1965; Sunday et al. 2011, 2014), although a few warm-adapted reptiles tolerate acute exposure to 47.5°C such as the desert iguana (*Dipsosaurus dorsalis*) (Cowles and Bogert 1944; Brattstrom 1965). Even so, most reptile and amphibian proteins do not lose function until they experience temperatures well above CT_{MAX}. For example, ribonucleases from three frog species were stable up to 85°C and maintained high activity at temperatures >50°C (Irie et al. 1998), lactate dehydrogenase in *Agama stellio* lizards maintained both stability and function up to 70°C (Al-Jassabi 2002), and alkaline phosphatase maintained high function up to 50°C in four lizard species (Licht 1964). Similarly, acute exposure to CT_{MAX} did not cause tissue damage or reduce function of serum glutamic-oxaloacetic or glutamic-pyruvic transaminases in Great Plains toads (*Anaxyrus cognatus*, Paulson and Hutchinson 1987) and 2.5-h exposure to temperatures just below CT_{MAX} had no effect on mitochondrial respiration or free-radical production in alligator lizards (*Elgaria coerulea* and *E. multicarinata*; Telemeco et al. 2017), implying no subcellular damage. That said, only one key component needs to lose function for the entire organism to become compromised. For example, the activity of myosin ATPase, a key enzyme for organismal muscle function, closely resembled whole-organism thermal performance curves and denatured at relatively low temperatures in eight lizard species corresponding closely to their respective CT_{MAX} (20% denatured between 37°C and 45.2°C; Licht 1964). Subcellular components such as ATPase could underlie thermal tolerance even though most components maintain function to higher temperature.

Given the complexities of subcellular interactions and the paucity of data for the thermal performance of subcellular components in reptiles and

amphibians, heat shock protein (HSP) production might better indicate whether subcellular-level components are challenged at sub-critical high temperatures. HSPs commonly act as molecular chaperones, maintaining protein structure and preventing aggregations of denatured proteins, and inducible variants are produced in response to cellular stress or damage (Fernando and Heikkila 2000; Kregel 2002; Daugaard et al. 2007). In diverse reptiles and amphibians, HSPs (particularly HSPA family members) are produced in response to sub-critical high temperatures, and production of HSPs can allow acclimation for increased thermal tolerance (Ulmasov et al. 1992; Fernando and Heikkila 2000; Zatespina et al. 2000; McMillan et al. 2011; Gao et al. 2014; Simoniello et al. 2016; Tedeschi et al. 2016). Moreover, patterns of HSP expression are correlated with thermal tolerance in lizards: warm-adapted species display higher constitutive HSP concentrations, and both initiate and maintain synthesis of HSPs to higher temperatures than more cold-adapted species (Ulmasov et al. 1992; Zatespina et al. 2000). This pattern of increased HSP production correlating with increases in thermal tolerance in reptiles and amphibians, along with observations for myosin ATPase in lizards, provides compelling evidence for subcellular-function loss playing a role in setting thermal limits, despite other subcellular components displaying little loss of function at relevant temperatures.

Organ-system mechanisms

Even though subcellular-level traits will be compromised at sufficiently high temperatures, higher-order systems might break down at lower temperatures and thus be proximally responsible for setting thermal tolerances (Pörtner 2001; 2002; Storch et al. 2014, but see Clark et al. 2013). The OCLTT hypothesis proposes that the highest organizational level in animals is the integrated cardiovascular and respiratory system because all tissues will be limited by their ability to acquire oxygen for respiration, and that this system is compromised by high temperatures prior to other systems (Pörtner 2001; 2002; Storch et al. 2014). However, similar to other terrestrial species (Klok et al. 2004; McCue and De Los Santos 2013; Verberk et al. 2016) and many fish (Clark et al. 2013; Gräns et al. 2014; Norin et al. 2014; Wang et al. 2014; Ern et al. 2016), evidence for the OCLTT mechanism underlying thermal tolerance in reptiles and amphibians is limited. Under the OCLTT hypothesis, maximal and resting rates of oxygen consumption are expected to converge as animals reach their physiological limits at high temperatures, thereby reducing aerobic scope and potentially inducing a short-term reliance on anaerobic respiration (Frederich and Pörtner 2000; Pörtner and Knust 2007; Elaison et al. 2011; Verberk et al. 2013, 2016; Table 1). Some evidence points to such a mechanism playing an important role in early animal evolution, notably in the transition to air breathing (Berner et al. 2007; Giomi et al. 2014; Teague et al. 2017). However, the few studies exposing animals to high temperatures and measuring indicators of aerobic and anaerobic respiration fail to find evidence for oxygen limitation in adult reptiles and amphibians (Carey 1979; Overgaard et al. 2012; Fobian et al. 2014; Gangloff et al. 2016; Telemeco et al. 2017). For example, oxygen consumption ($\dot{V}_{O_2}$) by pythons (*Python regius*) did not plateau at temperatures approaching CT_{MAX} either when at rest or during periods of high metabolic demand (Fobian et al. 2014, Fig. 1), and resting oxygen consumption in garter snakes (*Thamnophis elegans*) increased with temperature with no apparent limit when animals experienced near-lethal temperatures (Gangloff et al. 2016). Moreover, neither garter snakes (*T. elegans*) nor alligator lizards (*E. coerulea* and *E. multicarinata*) transitioned to anaerobic respiration when exposed to near-critical temperatures (Gangloff et al. 2016; Telemeco et al. 2017), despite snakes and lizards rapidly transitioning when oxygen availability is limited during exercise (reviewed in Gleeson 1991). Observations in amphibians are similar to those for reptiles. For example, oxygen consumption, arterial oxygen saturation, and the proportion of saturated hemoglobin did not plateau at high temperatures in active or resting cane toads (*Rhinella marina*), thus providing evidence for these toads' ability to maintain a positive aerobic power budget at near-critical temperatures (Seebacher and Franklin 2011; Overgaard et al. 2012; Winwood-Smith et al. 2015). In both the boreal toad (*Anaxyrus boreas*) and leopard frog (*Lithobates pipiens*), aerobic scope increased with temperature up to 30°C (Carey 1979). Whole-organism lactate concentration also increased with temperature, but there is no indication that either species becomes oxygen limited up to at least 30°C (Carey 1979). These results are in line with previous work showing that amphibians can maintain substantial aerobic scope at temperatures above active and preferred temperatures, although not necessarily at temperatures approaching T_{LETHAL} (Whitford 1973). While such studies provide strong evidence for adult reptiles and amphibians maintaining aerobic scope at high temperatures, we currently lack data on tissue and cellular oxygen supply, such as

Table 1 Experimental designs for testing aspects of the HMTL hypothesis in reptiles and amphibians with example studies

Experiment type: Factor manipulated	Manipulation	Dependent variable	What it demonstrates	Examples
Temperature	Temperature treatments or ramp in lab	Oxygen capacity parameters ($\dot{V}_{O_2}$, Active $\dot{V}_{O_2}$, alveolar/arterial P_{O_2}, heart rate), lactate production	Maintenance of aerobic scope at high temperatures; No evidence of transition to anaerobic respiration	Carey (1979), Seebacher and Franklin (2011), Overgaard et al. (2012), Fobian et al. (2014), Gangloff et al. (2016)
	Temperature treatments in lab	Skeletal muscle metabolome, mitochondrial function	No transition from aerobic to anaerobic metabolism or subcellular damage at high temperatures	Telemeco et al. (2017)
	Ex vivo temperature treatments	Enzyme activity	Temperature where subcellular components lose function	Licht (1964), Paulson and Hutchinson (1987), Irie et al. (1998), Al-Jassabi (2002)
	Temperature treatments in lab	HSP induction	Temperature that induces a subcellular-protection response	Ulmasov et al. (1992), Fernando and Heikkila (2000), Zatespina et al. (2000), McMillan et al. (2011), Gao et al. (2014), Simoniello et al. (2016), Tedeschi et al. (2016)
Ambient oxygen	Oxygen treatments in lab	Oxygen capacity parameters ($\dot{V}_{O_2}$, heart rate, alveolar/arterial P_{O_2}, ventilation rate)	Aerobic capacity is maintained under conditions of mild hypoxia, but is limited under extreme hypoxia	Boyer (1963, 1966), Withers and Hillman (1983), Pörtner et al. (1991), Branco et al. (1993), Wang et al. (1994)
	Oxygen treatments in lab	PBT	PBT is unaffected by mild hypoxia, but is reduced under extreme hypoxia	Hicks and Wood (1985), Branco et al. (1993), Cadena and Tattersall (2009)
Both ambient oxygen levels and temperature	Temperature and oxygen treatments in lab	Embryo development and survival	Hyperoxia increases survivorship while hypoxia reduces survivorship at high temperatures; Hypoxia reduces development, growth, and hatchling performance at high temperatures	Flewelling and Parker (2015), Liang et al. (2015), Smith et al. (2015)
	Temperature gradient and/or ramp and oxygen treatments in lab	CT_{MAX}, T_{GAPE}, T_{PANT}	Behavioral responses to high temperatures depend on ambient O_2, but only under extreme hypoxia	Dupre et al. (1986), Tattersall and Gerlach (2005), Shea et al. (2016)
	Transplant across altitudinal gradients within species' ranges; Manipulation of ambient O_2 in field; Temperature ramp	CT_{MAX}, T_{GAPE}, T_{PANT}	Behavioral responses to high temperatures depend on ambient O_2, but only under extreme hypoxia	DuBois et al. (2017)
Oxygen capacity	Hematocrit reduction	PBT	Reduced oxygen capacity affects temperature perception and animals choose lower temperatures	Wood (1990), Hicks and Wood (1985)
	Blood volume reduction	Oxygen capacity parameters (heart rate, alveolar/arterial P_{O_2}, ventilation rate)	Heart rate increases while ventilation rate is unchanged by reduced oxygen carrying capacity	Wang et al. (1994)
Observational	–	Quantification of CT_{MAX} across life stages	Oxygen capacity limits thermal tolerance at some developmental stages in larval anurans	Cupp (1980), Sherman (1980), Floyd (1983)
	–	Comparison of PBT, resting $\dot{V}_{O_2}$, Active $\dot{V}_{O_2}$,	PBT matches temperature of maximal aerobic scope in lizards	Wilson (1974)

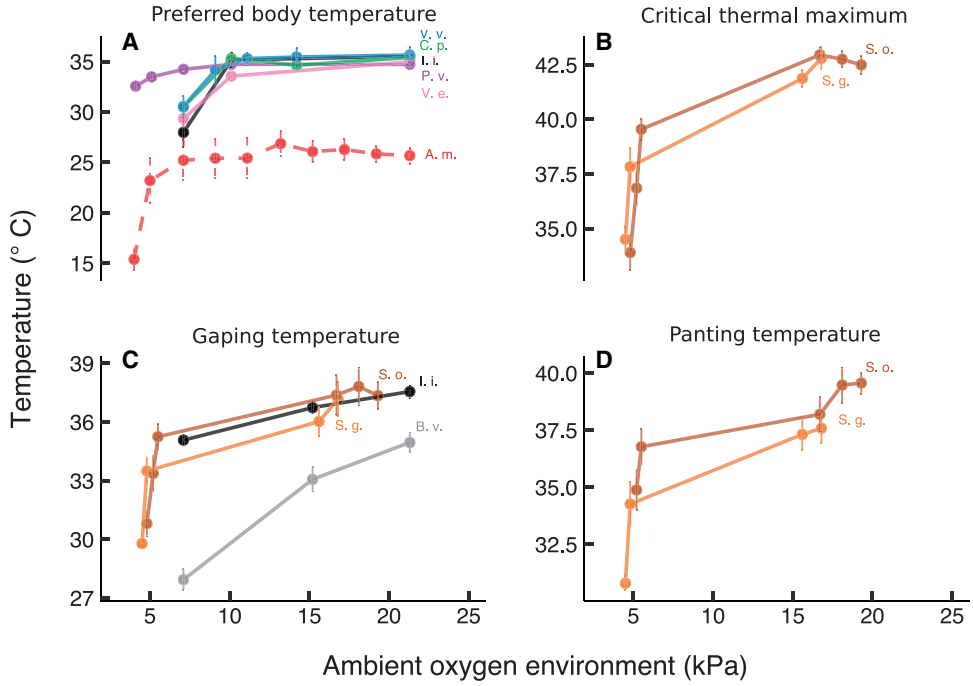

Fig. 2 Thermal preference and tolerance are unaffected by moderate hypoxia but reduced in extremely hypoxic environments (<10 kPa). This "broken-stick" pattern is apparent for (**A**) PBT (i.e. behavioral anapyrexia), (**B**) critical thermal maximum, (**C**) gaping temperature, and (**D**) panting temperature across species. Data are mostly available for lizards (solid lines) but PBT data are also available for alligator (dashed line). Lines between points are included to aid visualization, but connections between values at normoxia and hypoxia are likely nonlinear. Colors (online only) and initials denote species: A.m., *Alligator mississippiensis*; B.v., *Basiliscus vittatus*; C.p., *Ctenosaura pectinata*; I.i., *Iguana iguana*; P.v., *Pogona vitticeps*; S.g., *Sceloporus graciosus*; S.o., *Sceloporus occidentalis*; V.e., *Varanus exanthematicus*; V.v., *Varanus varius*. Data are means ± s.e.m. derived from the literature. See Supplementary Table S1 for data and citations.

venous P_{O_2}, which would more directly test the mechanisms described by the OCLTT hypothesis (Pörtner et al. 2017, 2018).

A second approach for testing the influence of oxygen capacity on thermal constraints is to manipulate oxygen availability and examine changes in thermal behavior or tolerance (Table 1). Under the OCLTT hypothesis, hypoxia is predicted to reduce thermal optima and tolerance limits (Smith et al. 2015; Verberk et al. 2016; DuBois et al. 2017), and this prediction is somewhat supported in reptiles and amphibians. For example, CT_{MAX}, preferred body temperature (PBT), panting temperature (T_{PANT}), and gaping temperature (T_{GAPE}) are reduced when diverse species are exposed to very-low oxygen environments (<10 kPa; mostly lizards examined; Hicks and Wood 1985; Dupre et al. 1986; Branco et al. 1993; Cadena and Tattersall 2009; Shea et al. 2016; DuBois et al. 2017; Fig. 2) and when hematocrit is experimentally reduced (only PBT examined; Hicks and Wood 1985; Wood 1990). Hypoxia-induced PBT reduction is a well-described phenomenon in ectotherms, termed "behavioral anapyrexia," that allows adaptive reduction of metabolic demand when oxygen is limited (Hicks and Wood 1985; Wood and Gonzales 1996; Steiner and Branco 2002; Hicks and Wang 2004), supporting the hypothesis that oxygen limitation influences thermal preference and possibly tolerance. Along with CT_{MAX}, reductions in T_{GAPE} and T_{PANT}, which provide an indication of perceived heat stress (Heatwole et al. 1973; Tattersall et al. 2006; DuBois et al. 2017), imply reduced thermal tolerance under hypoxia. Hypoxia also induces elevated heart rates and reduces both resting and active oxygen consumption ($\dot{V}_{O_2}$) in diverse species (Fig. 3), suggesting observed shifts in thermal tolerance and behavior are related to physiological limits of oxygen capacity (including diffusion and transport). Interestingly, only extreme hypoxia had the predicted effects on thermal tolerance and behavior, with levels of hypoxia within the range generally found in terrestrial environments having no effect (Figs. 2 and 3). Thus, it is not clear that the OCLTT mechanism will be generally relevant in nature.

In contrast to adult stages, naturalistic hypoxia reduces thermal performance and tolerance in eggs and larvae of reptiles and amphibians. Because

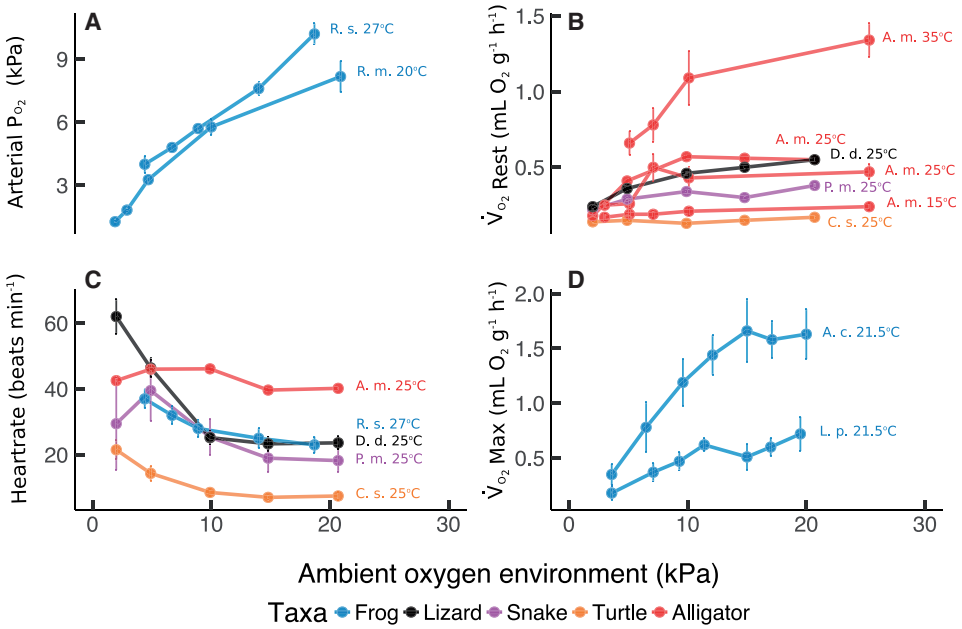

Fig. 3 As the environment becomes increasingly hypoxic, (**A**) arterial P_{O_2} decreases, whereas (**B**) resting metabolic rate, (**C**) heart rate, and (**D**) maximal metabolic rate are relatively unaffected until the environment becomes extremely hypoxic (<10 kPa), similar to thermal preference and tolerance (see Fig. 2 for comparison). Colors (online only) denote taxonomic group whereas initials denote species: A.c., *Anaxyrus cognatus* (formerly *Bufo cognatus*); A.m., *Alligator mississippiensis*; C.s., *Chelydra serpentina*; D.d, *Dipsosaurus dorsalis*; L.p., *Lithobates pipiens* (formerly *Rana pipiens*); P.m., *Pituophis melanoleucus*; R.m., *Rhinella marina* (formerly *Bufo marinus*); R.s., *Rhinella schneideri* (formerly *Bufo paracnemis*). A.c., L.p., R.s., and R.m. are frogs, D.d. is a lizard, P.m. is a snake, C.s. is a turtle, and A.m. is an alligator. Experimental temperature is given to the right of the species' initials. Data are means ± s.e.m. derived from the literature. See Supplementary Table S1 for data and citations.

amphibian eggs and larvae inhabit aqueous environments, environmental oxygen availability is reduced relative to terrestrial stages, thereby increasing the potential importance of the OCLTT mechanism similar to some fully aquatic ectotherms (e.g., Pörtner and Knust 2007; Verberk et al. 2013; Pörtner and Gutt 2016). Oxygen limitation is further exacerbated when species develop in small water bodies that can rapidly lose dissolved oxygen, such as ephemeral pools (Seymour and Bradford 1995; Sacerdote and King 2009), and because amphibian eggs are commonly produced within large gelatinous masses with low oxygen diffusion potential (Pinder and Friet 1994; Woods 1999; Sacerdote and King 2009). Although data are limited, these constraints appear to affect early stage amphibians congruent with oxygen limiting thermal tolerances. For example, amphibians that nest in warm water produce smaller eggs and egg masses to facilitate oxygen diffusion to embryos (Woods 1999; Sacerdote and King 2009), and aquatic larvae born into warm water (from aquatic or terrestrial eggs) are smaller and have lower metabolic demands (Kuramoto 1975; Rollinson and Rowe 2018). In oviparous reptiles, embryonic gas exchange occurs via passive diffusion across the shell into the chorioallantoic membrane, which is much less efficient than adult respiration (Vitt and Caldwell 2009). As predicted by the OCLTT hypothesis, thermal tolerance is reduced when developing embryos experience modest to extreme hypoxia (Flewelling and Parker 2015; Liang et al. 2015; Smith et al. 2015). For example, lower environmental P_{O_2} experienced at high elevations reduces T_{LETHAL} in plateau fence lizard (*Sceloporus tristichus*) embryos compared to P_{O_2} at sea level (Smith et al. 2015), although such modest hypoxia does not affect the thermal tolerance of adult congeners (*S. occidentalis* and *S. graciosus*; Shea et al. 2016; DuBois et al. 2017). Thus, available data suggest that reptiles and amphibians experience ontogenetic shifts in the proximate mechanism underlying thermal tolerance: early stages appear more affected by organ-system-level failures, such as the OCLTT mechanism, whereas later stages are more impacted by subcellular mechanisms.

An integrative framework: HMTL

Available evidence implies that neither failure of subcellular components nor oxygen and capacity

limitation are solely responsible for loss of performance at high temperatures in reptiles and amphibians. Rather, mechanisms across levels of organization appear to play partial, context-dependent roles. We propose a unified framework that combines subcellular mechanisms, the oxygen and capacity limited thermal tolerance (OCLTT) hypothesis, and the thermal performance curve (TPC) paradigm. We call this integrative framework "Hierarchical Mechanisms of Thermal Limitation" (HMTL, Fig. 1). Like the OCLTT hypothesis, we propose that oxygen diffusion and transport capacity largely shape thermal performance at sub-critical temperatures via effects of temperature on aerobic scope. However, the HMTL framework explicitly recognizes the importance of subcellular-level mechanisms and allows them to underlie absolute thermal limits, such as CT_{MAX} and T_{LETHAL}. Under this hypothesis, the relative importance of subcellular- and organ-system-level mechanisms on thermal tolerance is context dependent, but predictable. We propose that the HMTL framework explains diverse empirical results and can bring together decades of physiological studies in reptiles and amphibians.

Given that numerous models of thermal performance and tolerance have been proposed, we think that it is useful to state their similarities and differences with HMTL. The HMTL framework builds upon ideas developed for the OCLTT hypothesis proposed by Pörtner and colleagues (recently reviewed in Pörtner et al. 2017) and is similar to that put forth by Ern et al. (2016) for fishes (see Fig. 1 in Ern et al. 2016), but more formally incorporates the TPC paradigm and explicitly describes mechanism-dependent critical temperatures. Importantly, this hypothesis predicts conditions under which subcellular and organ-system failure will set thermal tolerance limits and when transitions are likely to occur. Our framework also differs importantly from the Multiple Performances—Multiple Optima (MPMO) framework put forth by Clark et al. (2013) for fishes. The MPMO suggests a single thermal limit set by an undefined mechanism but multiple, trait-specific optima that can dramatically differ from the temperature that maximizes aerobic scope (summarized in Fig. 7 of Clark et al. 2013). By contrast, we propose multiple potential mechanisms of thermal limitation but a single whole-organism thermal optimum for performance correlated with the temperature that maximizes aerobic scope (although this will be an integration across traits).

Figure 1 illustrates the HMTL framework. In the top row of panels, solid blue and red lines represent the effect of temperature on oxygen consumption during rest and activity, respectively. Assuming activity is maximal, aerobic scope is the difference between these lines. To illustrate this concept with empirical data, we derived these lines by fitting a generalized logistic function to data from Fobian et al. (2014) for pythons (*P. regius*) assuming the shape of the response curve is the same during rest and activity. Based on available data, the general shape of these curves is qualitatively similar across diverse taxa (e.g., Carey 1979; Frederich and Pörtner 2000; Overgaard 2012; Fobian et al. 2014; Ern et al. 2016) and we think that the predictions of the HMTL framework can be generalized beyond the specific data used to generate this illustration. The dotted blue and red lines depict exponential functions fit to these data and illustrate predicted oxygen demand/use if organisms were unconstrained by capacity limitations. The columns illustrate predicted effects of variable oxygen environments, and the bottom row displays TPCs predicted to result given relationships in the top row.

A central assumption of the HMTL framework is that critical thermal limits exist for both subcellular mechanisms (subcellular T_{CRIT}) and aerobic respiration (aerobic T_{CRIT}), and that organismal thermal limits are proximally caused by the lower T_{CRIT} (Fig. 1). The environment and oxygen-handling capacity of the organism will co-determine the hierarchy of these critical limits and thus their relative importance. The subcellular T_{CRIT} is the temperature at which key subcellular components begin to lose function, and should be insensitive to activity state or oxygen environment. By contrast, aerobic T_{CRIT} is the temperature at which oxygen capacity is maximized, and is affected by activity state and oxygen environment. For example, aerobic T_{CRIT} during rest will be higher than aerobic T_{CRIT} during activity because elevated O_2 demand during activity causes capacity limits to be reached more readily (Fig. 1). Moreover, both active- and resting-aerobic T_{CRIT} should be reduced during exposure to hypoxic environments (Fig. 1B, C). Any reduction in environmental oxygen availability below normoxia will reduce aerobic scope. However, CT_{MAX} will only be reduced if environmental hypoxia is sufficient to cause resting-aerobic T_{CRIT} to drop below subcellular T_{CRIT} (Fig. 1C). The environmental oxygen tension where resting-aerobic T_{CRIT} equals subcellular T_{CRIT} is $P_{CT_{MAX}}$ as defined by Ern et al. (2016).

By integrating T_{CRIT} values and aerobic scope, we can derive whole-organism TPCs (Fig. 1, bottom row). Because the active-aerobic T_{CRIT} is the lowest temperature where maximal metabolic rate can be achieved, it closely corresponds to the optimal

temperature for aerobic performance (whole-organism T_{OPT}) where aerobic scope is maximized. At temperatures below active-aerobic T_{CRIT}, we predict that performance increases with temperature proportional to aerobic scope. At temperatures above active-aerobic T_{CRIT}, performance will drop until CT_{MAX} is reached, but we predict that the shape of this drop will depend on which T_{CRIT} underlies CT_{MAX}. If resting-aerobic T_{CRIT} underlies CT_{MAX}, the TPC should be more symmetrical with performance and aerobic scope decreasing at a rate mirroring the increase (Fig. 1C). However, if subcellular T_{CRIT} underlies CT_{MAX}, the curve will be asymmetric with loss of performance occurring more rapidly the closer active-aerobic T_{CRIT} is to subcellular T_{CRIT} (Fig. 1A, B).

Assumptions, predictions, and evidence for HMTL in reptiles and amphibians

The HMTL framework produces numerous testable predictions, some of which can be addressed with available data, while others offer exciting avenues for future research. First, any environmental or organismal characteristics that reduce oxygen availability or capacity for oxygen utilization (e.g., aquatic respiration, reliance on cutaneous gas exchange, or life-stages such as eggs with reduced diffusion potential) should increase the probability of resting-aerobic T_{CRIT} underlying CT_{MAX}. This can explain oxygen capacity setting thermal limits in embryos (Smith et al. 2015) but not adults (Overgaard et al. 2012; Fobian et al. 2014; DuBois et al. 2017). Moreover, the transition between aerobic and subcellular T_{CRIT} predicted by HMTL explains extreme experimental hypoxia reducing thermal tolerance in adult reptiles and amphibians, while moderate hypoxia does not (Fig. 1C and Fig. 2B,D). Similarly, HMTL predicts that CT_{MAX} is lower when governed by oxygen limitation than when governed by subcellular mechanisms. Supporting this prediction, CT_{MAX} drops in tadpoles as oxygen demand increases with growth and when respiratory structures are compromised in late-stage metamorphs, but frequently elevates again in terrestrial adults presumably as a result of increased oxygen capacity (Cupp 1980; Sherman 1980; Floyd 1983).

Another important prediction of the HMTL hypothesis is that performance can be limited by oxygen exchange capacity at high temperatures even when CT_{MAX} and T_{LETHAL} are proximally set by subcellular mechanisms. Some interpretations of the OCLTT hypothesis similarly highlight limits on performance at pejus rather than critical temperatures (Pörtner and Knust 2007; Pörtner 2014; Verberk et al. 2016; Pörtner et al. 2017), but other workers suggest OCLTT should explain critical limits to be useful (i.e., Fobian et al. 2014; Smith et al. 2015; Ern et al. 2016; Verberk et al. 2016; DuBois et al. 2017). Under the HMTL framework, activity state is assumed to have no appreciable effect on oxygen capacity, although this may not hold in some highly aerobic taxa (e.g., Wang and Hicks 2004). The extent to which activity-induced increases in oxygen capacity affect thermal performance curves and limits is an important direction for future work. Generally however, individuals in metabolically-demanding states are expected to reach capacity limits at temperatures below those at which such limits are reached by resting individuals. Anaerobic respiration might compensate for short-term mismatches between energy demand and oxygen capacity (reviewed in Gleeson 1991; Fig. 1A, red shaded region), but aerobic constraints are predicted to reduce performance during long-term activity (e.g., digestion, reproduction, recovery; Jackson 2007; Pörtner et al. 2017).

In diverse species, active metabolic rate asymptotes despite increases in cardiovascular output, implying oxygen flux becomes limited at temperatures above active-aerobic T_{CRIT} (e.g., Bartholomew and Tucker 1963; Bennett and Licht 1972; Wilson 1974; Overgaard et al. 2012; Fobian et al. 2014). Furthermore, pulmonary diffusion capacity is limited by reduced plasma gas solubility and hemoglobin binding affinity at high temperatures (Wood and Moberly 1970; Kinney et al. 1977; Pough 1980; Jackson 2007; da Silva et al. 2013). Diffusion limitation of cutaneous gas exchange is also well established (reviewed in Burggren 1988; Wang 2011) and will be important in species that spend considerable time submerged (e.g. Ultsch 1973) or are lungless (e.g., Plethodontid salamanders; Whitford and Hutchison 1965; Spotilla 1972). Finally, increases in the products of anaerobic respiration, such as lactic acid, can lead to blood acidification and thereby further declines in blood oxygen affinity (Bennett 1973). The interaction of numerous factors at high temperatures reduces oxygen capacity, which in turn limits aerobic performance at high temperatures in reptiles and amphibians, regardless of the mechanism governing CT_{MAX} (further reviewed in Jackson 2007).

The HMTL framework predicts that whole-organism T_{OPT} is the temperature providing maximal aerobic scope, which is governed by active-aerobic T_{CRIT}. Thus, alleviating the limits that underlie active-aerobic T_{CRIT} should allow increased maximal performance (i.e., aerobic scope) and T_{OPT} (Fig. 1). Unfortunately, few data are available

comparing whole-organism T_{OPT} and aerobic scope in reptiles or amphibians. In the snake *P. regius*, whole-organism T_{OPT} and the temperature where aerobic scope is maximized appear to correspond (Fobian et al. 2014). In the toad *R. marina*, observations are more complex and suggest that the maintenance of aerobic scope to high temperatures could be independent of whole-organism T_{OPT}, at least in some cases. Hopping performance is maximized at ~30°C (Kearney et al. 2008) whereas aerobic scope can plateau at 30°C, but can also increase to at least 40°C depending on acclimation treatment (Overgaard et al. 2012). Additional data are needed to determine if acclimation elevates T_{OPT} for whole-organism performance similar to maximal aerobic scope in *R. marina*, as would be predicted by the HMTL framework.

Observations for PBT provide additional indirect evidence that whole-organism T_{OPT} corresponds to the temperature that maximizes aerobic scope in reptiles, but again data for amphibians are less clear. Generally, terrestrial ectotherms thermoregulate to within a narrow thermal range during activity if costs to thermoregulation are not prohibitively high (Huey 1982; Bauwens et al. 1995; Angilletta 2009; Kingsolver and Buckley 2015; Sears et al. 2016). Natural selection is predicted to shape thermal preference such that PBT corresponds to, or is slightly below, whole-organism T_{OPT} (Huey 1982; Bauwens et al. 1995; Angilletta et al. 2002; but see Huey and Bennett 1987). As predicted, PBT and temperature of maximum aerobic scope are highly concordant in lizards exposed to normoxic environments (Wilson 1974). On the other hand, in the boreal toad (*Anaxyrus boreas*), aerobic scope is maximal at 30°C whereas T_{PREF} is 24°C (Carey 1978, 1979). Interestingly, *A. boreas* and other anurans (*L. pipiens*, Carey 1979; *R. marina*, Overgaard et al. 2012) exhibit an increase in lactic acid production with temperatures above PBT in both resting and active animals. Increased lactic acid production indicates that high temperatures induce anaerobic respiration, even as aerobic scope is maintained, and therefore incur an oxygen debt for recovery. Further data are needed to assess whether the potential mismatch between maximal aerobic scope and T_{OPT} in anurans can be explained by animals balancing the increased costs of repaying oxygen debt resulting from increased anaerobic respiration at high temperatures with the benefits of concurrent increases in aerobic scope.

Assuming maximal aerobic scope, T_{OPT}, and PBT are linked as predicted by HMTL, they are not uniformly affected by experimental hypoxia as might initially be predicted. Only extreme hypoxia affects PBT (Fig. 2) whereas the temperature that maximizes aerobic scope and presumably whole-organism T_{OPT} is predicted to drop continuously with hypoxia (Fig. 1). This discrepancy might indicate that adult reptiles and amphibians cannot sense and respond to hypoxia-induced changes in aerobic scope in real time, which might be expected given that these animals evolved in terrestrial environments where oxygen availability is relatively stable within a lifetime. Thus, we propose that individuals choose the same body temperature regardless of the oxygen environment so long as basic metabolic demands are met, and thus predict that the "breakpoint" in Fig. 2A occurs when hypoxia causes resting-aerobic T_{CRIT} to fall below evolved PBT. Data comparing resting aerobic T_{CRIT} and PBT when oxygen environment or demand is manipulated are needed to test this prediction. Given the predicted relationships between aerobic scope, T_{OPT}, and PBT, we also expect species adapted to low-oxygen environments to have relatively lower T_{OPT} and PBT, or greater oxygen-handling capacity. However, covariation between temperature and oxygen with elevation make testing this prediction in terrestrial environments difficult.

In addition to further exploring the potential importance of resting- and active-aerobic T_{CRIT}, additional research is needed to identify the subcellular components that underlie subcellular T_{CRIT}. A subset of evolutionarily conserved components might constrain thermal tolerance across a diversity of taxa, or the components that are most important could be taxonomically specific. Currently, the data needed to differentiate these possibilities are not available. We think that measures of ATPase and HSP provide useful candidates for further exploration, but caution that focusing on a single or few potential indicators in isolation will likely provide a contorted view of subcellular limitation. Advances in "-omics" technologies, particularly differential expression RNAseq, metabolomics, and proteomics could provide much useful information about subcellular physiological function, and provide additional candidate molecules for detailed analysis (e.g., Verberk et al. 2013; Williams et al. 2014; Campbell-Staton et al. 2017; Telemeco et al. 2017). We recommend that experiments manipulating the thermal and oxygen environment of organisms endeavor to collect subcellular data as well as whole-organism performance data. Where possible, an integrative approach combining measurements of subcellular components and whole-organism performance will best illuminate the mechanisms that underlie tolerance and their interactions.

Finally, the HMTL framework makes predictions for how populations could be affected by global change. Numerous species are expanding or shifting their range to higher elevations in response to climate change-related temperature increases (e.g., Sinervo et al. 2010; Pincheira-Donoso et al. 2013; Pauchard et al. 2016). However, HMTL predicts that reduced oxygen partial pressures at high elevation will lower both T_{OPT} and maximal performance. Thus, species must seek cooler environments as they move to higher elevation to maintain optimal performance, and performance potential will go down regardless of thermal environment selected. An evolutionary change appears necessary for animals to seek out reduced body temperatures because moderate hypoxia does not affect PBT (Fig. 2). The HMTL framework also predicts that species with greater oxygen capacity will be more buffered from lost performance when exposed to increased environmental temperatures. Thus, oxygen capacity might be a prime target of natural selection as climates warm, even if it does not underlie CT_{MAX} or T_{LETHAL}. Finally, given the great diversity in modes of gas exchange, metabolic demands, and shifts across life-history stages in reptiles and amphibians, we emphasize the need to explore these hypotheses in a greater number and variety of taxa.

Conclusions

Available data suggest that both subcellular- and organ-system-level mechanisms shape thermal performance and tolerance in amphibians and reptiles. The HMTL framework that we propose describes how both mechanisms co-affect animals, with their relative importance driven by their respective T_{CRIT}. We think that the HMTL hypothesis improves upon current frameworks by explicitly removing the false dichotomy between subcellular mechanisms and oxygen limitation, identifying useful parameters for further research (subcellular T_{CRIT}, resting-aerobic T_{CRIT}, and active-aerobic T_{CRIT}), and describing how aerobic and subcellular limitations interact to affect TPCs. Moreover, HMTL appears to explain a wide range of initially perplexing observations in reptiles and amphibians such as reduced aerobic scope at high temperatures without aerobic failure at critical temperatures, extreme hypoxia affecting thermal tolerance and behavior in adults with no effect of moderate hypoxia, moderate hypoxia reducing thermal tolerance in embryos, and HSP and ATPase activity suggesting loss of subcellular function near critical temperatures. Still, the HMTL framework makes numerous predictions for which additional data are needed, including animals only reducing their body temperature when resting-aerobic T_{CRIT} drops below PBT, CT_{MAX} being lower when resting-aerobic T_{CRIT} is responsible than when subcellular T_{CRIT} is responsible, maximum aerobic capacity underlying whole-organism thermal optima, and reduced performance when species invade higher elevations without increased oxygen capacity. Moreover, virtually no data are available to address the potential importance of aerobic- or subcellular-T_{CRIT} as evolutionary constraints shaping the adaptive landscape. Further data better representing the diversity of reptile and amphibian taxa are needed both to understand the potential relevance of the HMTL mechanism in extant reptiles and amphibians, and how such a mechanism could have shaped the evolution of these animals. We think that many of the ideas that make up our HMTL framework are already widely accepted within the scientific community, and hope that explicitly describing them within a single framework with clear, testable predictions will facilitate further research. Although inspired by reptiles and amphibians, this integrated framework could have broad applicability across ectothermic animals. We look forward to continued investigation further integrating, refining, and testing these ideas across mechanisms and taxa.

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Supplementary data

Supplementary data are available at *ICB* online.

References

Al-Jassabi S. 2002. Purification and kinetic properties of skeletal muscle lactate dehydrogenase from the lizard *Agama stellio stellio*. Biochemistry (Moscow) 67:786–9.

Angilletta MJ. 2009. Thermal adaptation: a theoretical and empirical synthesis. New York (NY): Oxford University Press.

Angilletta MJ, Hill T, Robson MA. 2002. Is physiological performance optimized by thermoregulatory behavior?: A case study of the eastern fence lizard, *Sceloporus undulatus*. J Therm Biol 27:199–204.

Bartholomew GA, Tucker VA. 1963. Control of changes in body temperature, metabolism, and circulation by the Agamid lizard *Amphibolurus barbatus*. Physiol Zool 36:199–218.

Bauwens D, Garland T, Castilla AM, Van Damme R. 1995. Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioral covariation. Evolution 49:848–63.

Bennett AF. 1973. Blood physiology and oxygen transport during activity in two lizards *Varanus gouldii* and *Sauromalus hispidus*. Comp Biochem Physiol 46:673–90.

Bennett AF, Licht P. 1972. Anaerobic metabolism during activity in lizards. J Comp Physiol 81:277–88.

Berner RA, VandenBrooks JM, Ward PD. 2007. Oxygen and evolution. Science 316:557–8.

Boyer DR. 1963. Hypoxia: effects on heart rate and respiration in the snapping turtle. Science 140:813–14.

Boyer DR. 1966. Comparative effects of hypoxia on respiratory and cardiac function in reptiles. Physiol Zool 39:307–16.

Branco LGS, Pörtner HO, Wood SC. 1993. Interaction between temperature and hypoxia in the alligator. Am J Physiol 265:R1339–43.

Brattstrom BH. 1965. Body temperatures of reptiles. Am Midl Nat 73:376–422.

Buckley LB. 2008. Linking traits to energetics and population dynamics to predict lizard ranges in changing environments. Am Nat 171:E1–E19.

Buckley LB, Huey RB. 2016. How extreme temperatures impact organisms and the evolution of their thermal tolerance. Integr Comp Biol 56:98–109.

Burggren WW. 1988. Role of the central circulation in regulation of cutaneous gas exchange. Am Zool 28:985–98.

Cadena V, Tattersall GJ. 2009. Decreased precision contributes to the hypoxic thermoregulatory response in lizards. J Exp Biol 212:137–44.

Campbell-Staton SC, Cheviron ZA, Rochette N, Catchen J, Losos JB, Edwards SV. 2017. Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard. Science 357:495–8.

Carey C. 1978. Factors affecting body temperature of toads. Oecologia 35:197–219.

Carey C. 1979. Aerobic and anaerobic energy expenditure during rest and activity in montane *Bufo b. boreas* and *Rana pipiens*. Oecologia39:213–28.

Clark TD, Sandblom E, Jutfelt F. 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J Exp Biol 216:2771–82.

Colwell RK, Brehm G, Cardelús CL, Gilman AC, Longino JT. 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. Science 322:258–61.

Cowles RB, Bogert CM. 1944. A preliminary study of the thermal requirements of desert reptiles. Bull Am Mus Nat Hist 83:261–96.

Cupp PVJ. 1980. Thermal tolerance of five salientian amphibians during development and metamorphosis. Herpetologica 36:234–44.

da Silva GSF, Glass ML, Branco LGS. 2013. Temperature and respiratory function in ectothermic vertebrates. J Therm Biol 38:55–63.

Daugaard M, Rohde M, Jaattela M. 2007. The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. FEBS Lett 581:3702–10.

Deutsch CA, Tewksbury J, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc Natl Acad Sci U S A 105:6668–72.

Dillon ME, Woods HA, Wang G, Fey SB, Vasseur DA, Telemeco RS, Marshall K, Pincebourde S. 2016. Life in the frequency domain: the biological impacts of changes in climate variability at multiple time scales. Integr Comp Biol 56:14–30.

DuBois PM, Shea TK, Claunch NM, Taylor EN. 2017. Effects of oxygen on responses to heating in two lizard species sampled along an elevational gradient. J Therm Biol 68:170–6.

Dupre RK, Hicks JW, Wood SC. 1986. The effect of hypoxia on evaporative cooling thresholds of lizards. J Therm Biol 11:223–7.

Eliason EJ, Clark TD, Hague MJ, Hanson LM, Gallagher ZS, Jeffries KM, Gale MK, Patterson DA, Hinch SG, Farrell AP. 2011. Differences in thermal tolerance among sockeye salmon populations. Science 332:109–12.

Ern R, Norin T, Gamperl AK, Esbaugh AJ. 2016. Oxygen dependence of upper thermal limits in fishes. J Exp Biol 219:3376–83.

Fernando P, Heikkila JJ. 2000. Functional characterization of *Xenopus* small heat shock protein, Hsp30C: the carboxyl end is required for stability and chaperone activity. Cell Stress Chaperon 5:148–59.

Fields PA. 2001. Review: protein function at thermal extremes: balancing stability and flexibility. Comp Biochem Physiol A 129:417–31.

Flewelling S, Parker SL. 2015. Effects of temperature and oxygen on growth and differentiation of embryos of the ground skink, *Scincella lateralis*. J Exp Zool 323:445–55.

Floyd RB. 1983. Ontogenetic change in the temperature tolerance of larval *Bufo marinus* (Anura: bufonidae). Comp Biochem Physiol 75:267–71.

Fobian D, Overgaard J, Wang T. 2014. Oxygen transport is not compromised at high temperature in pythons. J Exp Biol 217:3958–61.

Frederich M, Pörtner HO. 2000. Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. Am J Physiol 279:R1531–8.

Gangloff EJ, Holden KG, Telemeco RS, Baumgard LH, Bronikowski AM. 2016. Hormonal and metabolic responses to upper temperature extremes in divergent life-history ecotypes of a garter snake. J Exp Biol 219:2944–54.

Gao J, Zhang W, Dang W, Mou Y, Gao Y, Sun BJ, Du WG. 2014. Heat shock protein expression enhances heat tolerance of reptile embryos. Proc Biol Sci 281:20141135.

Giomi F, Fusi M, Barausse A, Mostert B, Pörtner HO, Cannicci S. 2014. Improved heat tolerance in air drives the recurrent evolution of air-breathing. Proc Biol Sci 281:20132927.

Glass ML, Johansen K, Abe AS. 1981. Pulmonary diffusing capacity in reptiles (relations to temperature and O_2-uptake). J Comp Physiol 142:509–14.
Gleeson TT. 1991. Patterns of metabolic recovery from exercise in amphibians and reptiles. J Exp Biol 160:187–207.
Gräns A, Jutfelt F, Sandblom E, Jönsson E, Wiklander K, Seth H, Olsson C, Dupont S, Ortega-Martinez O, Einarsdottir I, et al. 2014. Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO_2 in Atlantic halibut. J Exp Biol 217:711–17.
Gunderson AR, Stillman JH. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc R Soc B 282:20150401.
Heatwole H, Firth B, Webb G. 1973. Panting thresholds of lizards-I. Some methodological and internal influences on the panting threshold of an agamid, *Amphibolurus muricatus*. Comp Biochem Physiol A 46:799–826.
Helmuth B, Kingsolver JG, Carrington E. 2005. Biophysics, physiological ecology, and climate change: does mechanism matter? Annu Rev Physiol 67:177–201.
Hicks JW, Wang T. 2004. Hypometabolism in reptiles: behavioural and physiological mechanisms that reduce aerobic demands. Resp Physiol Neurobiol 141:261–71.
Hicks JW, Wood SC. 1985. Temperature regulation in lizards: effects of hypoxia. Am Physiol 248:R595–600.
Hochachka PW, Somero GN. 2002. Biochemical adaptation: mechanism and process in physiological evolution. Oxford (UK): Oxford University Press.
Huey RB. 1982. Temperature, physiology, and the ecology of reptiles. In: Gans C, Pough FH, editors. Biology of the reptilia. New York (NY): Academic Press. p. 25–74.
Huey RB, Bennett AF. 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. Evolution 41:1098–115.
Huey RB, Berrigan D. 2001. Temperature, demography, and ectotherm fitness. Am Nat 158:204–10.
Huey RB, Kearney MR, Krockenberger A, Holtum JAM, Jess M, Williams SE. 2012. Predicting organismal vulnerability to climate warming: roles of behavior, physiology and adaptation. Philos Trans R Soc B 367:1665–79.
Huey RB, Losos JB, Moritz C. 2010. Are lizards toast? Science 328:832–3.
Huey RB, Stevenson RD. 1979. Integrating thermal physiology and ecology of ectotherms: discussion of approaches. Am Zool 19:357–66.
Hutchison VH, Whitford WG, Kohl M. 1968. Relation of body size and surface area to gas exchange in anurans. Physiol Zool 41:65–85.
Irie M, Nitta K, Nonaka T. 1998. Biochemistry of frog ribonucleases. Cell Mol Life Sci 54:775–84.
Jackson DC. 2007. Temperature and hypoxia in ectothermic tetrapods. J Therm Biol 32:125–33.
Jutfelt F, Gräns A, Jönsson E, Wiklander K, Seth H, Olsson C, Dupont S, Ortega-Martinez O, Sundell K, Axelsson M. 2014. Response to 'How and how not to investigate the oxygen and capacity limitation of thermal tolerance (OCLTT) and aerobic scope - remarks on the article by Gräns et al.'. J Exp Biol 217:4433–5.
Jutfelt F, Norin T, Ern R, Overgaard J, Wang T, McKenzie DJ, Lefevre S, Nilsson GE, Metcalfe NB, Hickey AJR, et al. 2018. Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. J Exp Biol 221: jeb169615.
Kearney M. 2012. Metabolic theory, life history and the distribution of a terrestrial ectotherm. Funct Ecol 26:167–79.
Kearney M. 2013. Activity restriction and the mechanistic basis for extinctions under climate warming. Ecol Lett 16:1470–9.
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 WP. 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. Ecol Lett 12:334–50.
Kingsolver JG, Buckley LB. 2015. Climate variability slows evolutionary responses of *Colias* butterflies to recent climate change. Proc Biol Sci 282:20142470.
Kingsolver JG, Woods HA. 2016. Beyond thermal performance curves: modeling time-dependent effects of thermal stress on ectotherm growth rates. Am Nat 187:283–94.
Kinney JL, Matsuura DT, White FN. 1977. Cardiorespiratory effects of temperature in the turtle, *Pseudemys floridana*. Resp Physiol 31:309–25.
Klok CJ, Sinclair BJ, Chown SL. 2004. Upper thermal tolerance and oxygen limitation in terrestrial arthropods. J Exp Biol 207:2361–70.
Kregel K. 2002. Heat shock proteins: modifying factors in physiological stress response and acquired thermotolerance. J Appl Physiol 92:2177–86.
Kuramoto M. 1975. Adaptive significance in oxygen consumption of frog embryos in relation to the environmental temperatures. Comp Biochem Physiol 52:59–62.
Levy O, Buckley LB, Keitt TH, Smith CD, Boateng KO, Kumar DS, Angilletta MJ. 2015. Resolving the life cycle alters expected impacts of climate change. Proc Biol Sci 282:20150837.
Liang L, Sun BJ, Ma L, Du WG. 2015. Oxygen-dependent heat tolerance and developmental plasticity in turtle embryos. J Comp Physiol B 185:257–63.
Licht P. 1964. The temperature dependence of myosin-adenosinetriphosphatase and alkaline phosphatase in lizards. Comp Biochem Physiol 12:331–40.
Lucassen M, Koschnick N, Eckerle LG, Pörtner HO. 2006. Mitochondrial mechanisms of cold adaptation in cod (*Gadus morhua* L.) populations from different climatic zones. J Exp Biol 209:2462–71.
Malishev M, Bull CM, Kearney MR. 2018. An individual-based model of ectotherm movement integrating metabolic and microclimatic constraints. Methods Ecol Evol 9:472–89.
McCue MD, De Los Santos R. 2013. Upper thermal limits of insects are not the result of insufficient oxygen delivery. Physiol Biochem Zool 86:257–65.
McMillan DM, Irschick DJ, Rees BB. 2011. Geographic variation in the effects of heat exposure on maximum sprint speed and Hsp70 expression in the western fence lizard *Sceloporus occidentalis*. Physiol Biochem Zool 84:573–82.
Norin T, Malte H, Clark TD. 2014. Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. J Exp Biol 217:244–51.

Overgaard J, Andersen JL, Findsen A, Pedersen PB, Hansen K, Ozolina K, Wang T. 2012. Aerobic scope and cardiovascular oxygen transport is not compromised at high temperatures in the toad *Rhinella marina*. J Exp Biol 215:3519–26.

Pauchard A, Milbau A, Albihn A, Alexander J, Burgess T, Daehler C, Englund G, Essl F, Evengård B, Greenwood GB, et al. 2016. Non-native and native organisms moving into high elevation and high latitude ecosystems in an era of climate change: new challenges for ecology and conservation. Biol Invasion 18:345–53.

Paulson BK, Hutchison VH. 1987. Blood changes in *Bufo cognatus* following acute heat stress. Comp Biochem Physiol 87:461–6.

Pincheira-Donoso D, Tregenza T, Witt MJ, Hodgson DJ. 2013. The evolution of viviparity opens opportunities for lizard radiation but drives it into a climate cul-de-sac. Global Ecol Biogeogr 2013:1–11.

Pinder AW, Friet SC. 1994. Oxygen transport in egg masses of the amphibians *Rana sylvatica* and *Ambystoma maculatum*: convection, diffusion, and oxygen production by algae. J Exp Biol 197:17–30.

Pörtner HO. 2014. How and how not to investigate the oxygen and capacity limitation of thermal tolerance (OCLTT) and aerobic scope - remarks on the article by Gräns et al. J Exp Biol 217:4432–3.

Pörtner HO. 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88:137–46.

Pörtner HO. 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp Biochem Physiol A 132:739–61.

Pörtner HO, Bock C, Mark FC. 2017. Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. J Exp Biol 220:2685–96.

Pörtner HO, Gutt J. 2016. Impacts of climate variability and change on (marine) animals: physiological underpinnings and evolutionary consequences. Integr Comp Biol 56:31–44.

Pörtner HO, Knust R. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315:95–7.

Pörtner HO, MacLatchy LM, Toews DP. 1991. Metabolic responses of the toad *Bufo marinus* to environmental hypoxia: an analysis of the critical P_{O2}. Physiol Zool 64:836–49.

Pough FH. 1980. Blood oxygen transport and delivery in reptiles. Am Zool 20:173–85.

Rezende EL, Castañeda LE, Santos M, Fox C. 2014. Tolerance landscapes in thermal ecology. Funct Ecol 28:799–809.

Rohr JR, Raffel TR. 2010. Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. Proc Natl Acad Sci U S A 107:8269–74.

Rollinson N, Rowe L. 2018. Oxygen limitation at the larval stage and the evolution of maternal investment per offspring in aquatic environments. Am Nat 191 published online (doi:10.1086/696857).

Sacerdote AB, King RB. 2009. Dissolved oxygen requirements for hatching success of two ambystomatid salamanders in restored ephemeral ponds. Wetlands 29:1202–13.

Schulte PM. 2015. The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. J Exp Biol 218:1856–66.

Sears MW, Angilletta MJJ, Schuler MS, Borchert J, Dilliplane KF, Stegman M, Rusch TW, Mitchell WA. 2016. Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. Proc Natl Acad Sci U S A 113:10595–600.

Seebacher F, Franklin CE. 2011. Physiology of invasion: cane toads are constrained by thermal effects on physiological mechanisms that support locomotor performance. J Exp Biol 214:1437–44.

Seebacher F, White CR, Franklin CE. 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. Nat Clim Change 5:61–6.

Seymour RS, Bradford DF. 1995. Respiration of amphibian eggs. Physiol Zool 68:1–25.

Shea TK, DuBois PM, Claunch NM, Murphey NE, Rucker KA, Brewster RA, Taylor EN. 2016. Oxygen concentration affects upper thermal tolerance in a terrestrial vertebrate. Comp Biochem Physiol A 199:87–94.

Sheldon KS, Dillon ME. 2016. Beyond the mean: biological impacts of cryptic temperature change. Integr Comp Biol 56:110–19.

Sherman E. 1980. Ontogenetic change in thermal tolerance of the toad *Bufo woodhousii fowleri*. Comp Biochem Physiol 65:227–30.

Simoniello P, Esposito MG, Trinchella F, Motta CM, Scudiero R. 2016. Alterations in brain morphology and HSP70 expression in lizard embryos exposed to thermal stress. C R Biol 339:380–90.

Sinervo B, Méndez-de-la-Cruz F, Miles DB, Heulin B, Bastiaans E, Cruz MV-S, Lara-Resendiz R, Martinez-Méndez N, Calderón-Espinosa ML, Meza-Lázaro RN. 2010. Erosion of lizard diversity by climate change and altered thermal niches. Science 328:894–9.

Smith C, Telemeco RS, Angilletta MJ, VandenBrooks JM. 2015. Oxygen supply limits the heat tolerance of lizard embryos. Biol Lett 11:20150113.

Snyder GK, Weathers WW. 1975. Temperature adaptations in amphibians. Am Nat 109:93–101.

Spotila JR. 1972. Role of temperature and water in the ecology of lungless salamanders. Ecol Monogr 42:95–125.

Steiner AA, Branco LG. 2002. Hypoxia-induced anapyrexia: implications and putative mediators. Annu Rev Physiol 64:263–88.

Storch D, Menzel L, Frickenhaus S, Pörtner HO. 2014. Climate sensitivity across marine domains of life: limits to evolutionary adaptation shape species interactions. Global Change Biol 20:3059–67.

Sunday JM, Bates AE, Dulvy NK. 2011. Global analysis of thermal tolerance and latitude in ectotherms. Proc Biol Sci 278:1823–30.

Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, Huey RB. 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proc Natl Acad Sci U S A 111: 5610–15.

Tattersall GJ, Cadena V, Skinner MC. 2006. Respiratory cooling and thermoregulatory coupling in reptiles. Respir Physiol Neurobiol 154:302–18.

Tattersall GJ, Gerlach RM. 2005. Hypoxia progressively lowers thermal gaping thresholds in bearded dragons, *Pogona vitticeps*. J Exp Biol 208:3321–30.

Teague C, Youngblood JP, Ragan K, Angilletta MJ, Jr, VandenBrooks JM. 2017. A positive genetic correlation between hypoxia tolerance and heat tolerance supports a controversial theory of heat stress. Biol Lett 13:20170309.

Tedeschi JN, Kennington WJ, Tomkins JL, Berry O, Whiting S, Meekan MG, Mitchell NJ. 2016. Heritable variation in heat shock gene expression: a potential mechanism for adaptation to thermal stress in embryos of sea turtles. Proc R Soc B 283:20152320.

Telemeco RS. 2014. Immobile and mobile life-history stages have different thermal physiologies in a lizard. Physiol Biochem Zool 87:203–15.

Telemeco RS, Gangloff EJ, Cordero GA, Polich RL, Bronikowski AM, Janzen FJ. 2017. Physiology at near-critical temperatures, but not critical limits, varies between two lizard species that partition the thermal environment. J Anim Ecol 86:1510–22.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, De Siqueira MF, Grainger A, Hannah L, et al. 2004. Extinction risk from climate change. Nature 427:145–8.

Ulmasov KA, Shammakov S, Karaev K, Evgen'ev MB. 1992. Heat shock proteins and thermoresistance in lizards. Proc Natl Acad Sci U S A 89:1666–70.

Ultsch GR. 1973. A theoretical and experimental investigation of the relationship between metabolic rate, body size, and oxygen exchange capacity. Respir Physiol 18:143–60.

Vasseur DA, DeLong JP, Gilbert B, Greig HS, Harley CD, McCann KS, Savage V, Tunney TD, O'Connor MI. 2014. Increased temperature variation poses a greater risk to species than climate warming. Proc Biol Sci 281:20132612.

Verberk WCEP, Leuven RSEW, van der Velde G, Gabel F, Overgaard J. 2018. Thermal limits in native and alien freshwater peracarid Crustacea: the role of habitat use and oxygen limitation. Funct Ecol published online (doi:10.1111/1365-2435.13050).

Verberk WCEP, Overgaard J, Ern R, Bayley M, Wang T, Boardman L, Terblanche JS. 2016. Does oxygen limit thermal tolerance in arthropods? A critical review of current evidence. Comp Biochem Physiol A 192:64–78.

Verberk WCEP, Sommer U, Davidson RL, Viant MR. 2013. Anaerobic metabolism at thermal extremes: a metabolomic test of the oxygen limitation hypothesis in an aquatic insect. Integr Comp Biol 53:609–19.

Vitt LJ, Caldwell JP. 2009. Herpetology: an introductory biology of amphibians and reptiles. New York (NY): Academic Press.

Wang T. 2011. Gas exchange in frogs and turtles: how ectothermic vertebrates contributed to solving the controversy of pulmonary oxygen secretion. Acta Physiol 202:593–600.

Wang T, Branco LGS, Glass ML. 1994. Ventilatory responses to hypoxia in the toad *Bufo paracnemis* before and after a decrease in haemoglobin oxygen-carrying capacity. J Exp Biol 186:1–8.

Wang T, Hicks JW. 2004. Why savannah monitor lizards hyperventilate during activity: a comparison of model predictions and experimental data. Respir Physiol Neurobiol 144:251–61.

Wang T, Lefevre S, Iversen NK, Findorf I, Buchanan R, McKenzie DJ. 2014. Anaemia only causes a small reduction in the upper critical temperature of sea bass: is oxygen delivery the limiting factor for tolerance of acute warming in fishes? J Exp Biol 217:4275–8.

Whitford WG. 1973. The effects of temperature on respiration in the amphibia. Am Zool 13:505–12.

Whitford WG, Hutchison VH. 1965. Gas exchange in salamanders. Physiol Zool 38:228–42.

Williams CM, Buckley LB, Sheldon KS, Vickers M, Portner HO, Dowd WW, Gunderson AR, Marshall KE, Stillman JH. 2016. Biological impacts of thermal extremes: mechanisms and costs of functional responses matter. Integr Comp Biol 56:73–84.

Williams CM, Watanabe M, Guarracino MR, Ferraro MB, Edison AS, Morgan TJ, Boroujerdi AF, Hahn DA. 2014. Cold adaptation shapes the robustness of metabolic networks in *Drosophila melanogaster*. Evolution 68:3505–23.

Wilson KJ. 1974. The relationship of oxygen supply for activity to body temperature in four species of lizards. Copeia 1974:920–34.

Winwood-Smith HS, Alton LA, Franklin CE, White CR. 2015. Does greater thermal plasticity facilitate range expansion of an invasive terrestrial anuran into higher latitudes? Conserv Phys 3. doi: 10.1093/conphys/cov010.

Withers PC, Hillman SS. 1983. The effects of hypoxia on pulmonary function and maximal rates of oxygen consumption in two anuran amphibians. J Comp Physiol B 152:125–9.

Wood SC. 1990. Effect of hematocrit on behavioral thermoregulation of the toad *Bufo marinus*. Am J Physiol 258:R848–51.

Wood SC, Gonzales R. 1996. Hypothermia in hypoxic animals: mechanisms, mediators, and functional significance. Comp Biochem Physiol 113:37–43.

Wood SC, Moberly WR. 1970. The influence of temperature on the respiratory properties of iguana blood. Respir Physiol 10:20–9.

Woods HA. 1999. Egg-mass size and cell size: effects of temperature on oxygen distribution. Am Zool 39:244–52.

Zatsepina OG, Ulmasov KA, Beresten SF, Molodtsov VB, Rybtsov SA, Evgen'ev MB. 2000. Thermotolerant desert lizards characteristically differ in terms of heat-schock system regulation. J Exp Biol 203:1017–25.