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COMMENTARY

Dissecting cause from consequence: a systematic approach to thermal limits

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ABSTRACT

Thermal limits mark the boundaries of ectotherm performance, and are increasingly appreciated as strong correlates and possible determinants of animal distribution patterns. The mechanisms setting the thermal limits of ectothermic animals are under active study and rigorous debate as we try to reconcile new observations in the lab and field with the knowledge gained from a long history of research on thermal adaptation. Here, I provide a perspective on our divided understanding of the mechanisms setting thermal limits of ectothermic animals. I focus primarily on the fundamental differences between high and low temperatures, and how animal form and environment can place different constraints on different taxa. Together, complexity and variation in animal form drive complexity in the interactions within and among levels of biological organization, creating a formidable barrier to determining mechanistic cause and effect at thermal limits. Progress in our understanding of thermal limits will require extensive collaboration and systematic approaches that embrace this complexity and allow us to separate the causes of failure from the physiological consequences that can quickly follow. I argue that by building integrative models that explain causal links among multiple organ systems, we can more quickly arrive at a holistic understanding of the varied challenges facing animals at extreme temperatures.

KEY WORDS: Comparative physiology, Critical thermal limits, Ecophysiology, Ectotherm performance

Introduction

At the edges of the range of temperatures that animals can tolerate lie their critical thermal limits – the temperatures at which fitness or performance (i.e. some fitness-related measure) reaches zero. This simple idea of a thermal limit as a single trait or measure is attractive, but measurement and interpretation of thermal limits has proven challenging, and our understanding of thermal limits has grown beyond this tidy definition. Thermal limitation can be, and is being, measured using multiple organismal phenotypes that can all occur at different temperatures, such as decreased reproductive output, characteristic changes in behaviour, an inability to maintain body position or complete neuromuscular collapse. Animals that experience these effects of heating or cooling can suffer from reduced or zero fitness; individuals exposed to temperatures beyond some of these limits often cannot escape those conditions, and with time can suffer from physiological collapse leading to injury and death. These ultimate consequences of exposure to thermal extremes are often themselves also regarded as measures of thermal limits (Cowles and Bogert, 1944; Lutterschmidt and Hutchison, 1997). To complicate matters further, any one of these ways of measuring thermal limits can be measured across different time scales (i.e. rapid versus slow temperature change or acute versus chronic exposure).

Although their definition and measurement can prove challenging, understanding thermal limits is clearly of great importance; as animals exposed to temperatures limiting behaviour, activity and survival either are, or are on their way to being, 'ecologically dead', thermal limits hold promise of great relevance to our understanding of animal distribution, ecology and policy. Given the critical and diverse roles that animals play in our lives, and a justified concern for the effects of anthropogenic climate change on animal diversity and abundance (Bozinovic and Pörtner, 2015), for example, the thermal limits of a broad range of taxa are a current focus of research.

Interest in thermal limits can be conceptually divided into studies that focus on the utility of these traits and those that seek to understand their underlying mechanisms. On the one hand, thermal limits are simple traits that can conveniently inform our understanding of how thermal adaptation shapes ectotherm performance (Deutsch et al., 2008; Overgaard et al., 2014; Payne et al., 2016; Pecl et al., 2017; Pörtner, 2010; Stillman, 2003; Sunday et al., 2011, 2014; Walther et al., 2002). On the other hand, thermal limits are complex organismal traits in need of mechanistic explanation (Huey et al., 2012; Overgaard and MacMillan, 2017; Pörtner and Farrell, 2008).

The search for mechanisms underlying thermal limits has resulted in integrative models that aim to place the limits of thermal tolerance within a specific physiological context (through the lens of, for example, aerobic metabolism or ion and water homeostasis; Fig. 1). The strength of these models is that they provide attractive avenues for understanding organismal failure. The danger of these models, however, is that there is a natural tendency to regard them as 'general rules' that can explain both upper and lower thermal limits and can be applied to all animals, regardless of their natural history or taxonomy. By hoping for such generalities, we risk ignoring other mechanisms that may be of equal or greater importance (or of importance only for specific groups); a resulting research bias toward a singular explanation for thermal limits could hamper meaningful progress during a period of global urgency.

In this Commentary, I begin by highlighting the varied ways in which we measure organismal thermal limits, and discuss the dangers of treating different measures of thermal limits as conceptually equivalent. I then provide an overview of two integrative models for understanding thermal limits that emerged from studies on different taxa. Using these models as examples, I argue that integrative models that emphasize causal relationships, when combined with a systematic research approach, can provide us with an efficient path toward a thorough and predictive understanding of what sets the limits of ectotherm performance.

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Journal of Experimental Biology (2019) 222, jeb191593. doi:10.1242/jeb.191593

Glossary

Aerobic scope
The difference between resting metabolic rate and maximal metabolic rate (e.g. induced through exercise).
Critical thermal maximum (CT_{max})
High temperature at which performance (broadly interpreted) is equal to zero. Also generally referred to as a high temperature at which animals lose the ability to stand or maintain equilibrium.
Critical thermal minimum (CT_{min})
Low temperature at which performance (broadly interpreted) is equal to zero. Also generally referred to as a low temperature at which animals lose the ability to stand or maintain equilibrium.
Hyperkalaemia
High extracellular concentrations of K^+.
Hypoxaemia
Low oxygen concentrations in the blood or haemolymph.
Ionoregulatory collapse
A model of the mechanisms setting limits to performance at low temperatures. According to this model, lower thermal limits are tied to the inability to maintain ion and water balance in the cold.
Loss of equilibrium (LOE)
The inability to maintain upright posture. One common method of measuring critical thermal limits in fishes.
Multiple performances–multiple optima (MPMO) hypothesis
The hypothesis that different physiological functions have different thermal optima, and that contributions of multiple performance traits to organismal performance can vary among species.
Oxygen and capacity limitation of thermal tolerance (OCLTT)
A model of the mechanisms setting limits to thermal performance. According to this model, limits of thermal tolerance are set by temperature effects on aerobic scope.
Thermal performance curve
Two-dimensional mathematical equations that describe the effects of temperature on some measure of organismal performance.

Discriminating among measures of thermal limitation: what limits matter?

Critical thermal limits (CT_{min} and CT_{max}; see Glossary) are the temperatures at which some measure of performance is equal to zero. This simple concept fits nicely into a schema of thermal performance curves (see Glossary) as a two-dimensional relationship between an organism's physiological performance and its body temperature (T_b; Fig. 1A). If performance is regarded as an indirect measure of fitness, fitness is equal to zero when T_b extends below CT_{min} or above CT_{max} (Deutsch et al., 2008; Sinclair et al., 2016). There is evidence from some taxa (e.g. insects) that these critical thermal limits to activity and survival are of great importance to animal fitness and thus biogeography (Marshall and Sinclair, 2012; Overgaard et al., 2014). However, other studies (e.g. on marine vertebrates and invertebrates) have led authors to argue that the long-term fitness effects of milder temperatures (e.g. acting directly on animal growth or reproduction, or through impairments in biotic interactions) are more ecologically relevant (Healy and Schulte, 2012; Pörtner and Knust, 2007; Pörtner et al., 2017). One of these views may be correct for any species operating within a given environment, or both may be correct in some scenarios (e.g. in a situation where both predominant temperatures and sudden heat waves or cold snaps influence fitness). The question of what limits matter most to fitness is perhaps the one that most urgently needs to be resolved if we hope to link physiological knowledge to our understanding of animal distribution and abundance in the natural world, precisely because the traits we choose to focus on may emerge as ecologically unimportant.

In addition to its broader definition, the term critical thermal limit (e.g. CT_{min} or CT_{max}) is used regularly to refer specifically to a temperature causing motor defects in a wide variety of animals. However, even when a 'specific' thermal limit has been selected for investigation, methods of quantifying these limits often vary depending on the experimenter and the species studied. For example, the CT_{max} of fish is often defined as the temperature at which a fish experiences a loss of equilibrium (LOE; see Glossary), which could be the point of uncontrolled and disorganized swimming (Morgan et al., 2018) or the point where the ability to remain upright is lost and not regained (Becker and Genoway, 1979). This second measure in fish generally agrees with the loss of the righting response that is typically measured in reptiles and amphibians (Dayananda et al., 2017). Although these small differences in methodology may seem insignificant, if the goal is to understand the mechanisms of physiological failure occurring at thermal limits, how these limits are measured really matters. This is in part because seemingly equivalent measures of thermal tolerance can be mediated by different underlying mechanisms (Beitinger et al., 2000; MacMillan and Sinclair, 2011a; Overgaard and MacMillan, 2017), and because many studies downplay the critical importance of time and functional responses as factors in measuring the limits of performance (e.g. the interplay between acclimation responses and development of injuries during thermal ramping assays; Allen et al., 2016; Kingsolver and Woods, 2016; Mora and Maya, 2006; Peck et al., 2009; Terblanche et al., 2007; Williams et al., 2016).

Insect chill coma serves as a good example of how seemingly equivalent thermal limit measurements can be quite different. The term 'chill coma' has historically been given at least four different meanings in the insect literature, and has been muddled with the term CT_{min} (Hazell and Bale, 2011). For example, chill coma has been used to mean a lack of spontaneous movement, a loss of coordination or a fully comatose state (i.e. a complete inability to move). Measuring any one of these 'types' of an insect's lower thermal limit can provide a simple means of comparing thermal tolerance among species, populations or treatment groups, as an animal that does not effectively move about its environment cannot find food, shelter or mates (meaning its fitness is effectively zero). From a physiological perspective, however, it has become evident that these different methods of quantifying failure may, in fact, be measuring entirely different physiological end points (MacMillan et al., 2012; Robertson et al., 2017). While a lack of spontaneous movement may reflect higher-level information processing (i.e. a measure of temperature effects on behaviour), a loss of coordination may result from spreading depolarization in the central nervous system (Robertson et al., 2017), and a complete absence of movement may more effectively quantify a later failure of muscle L-type Ca^{2+} channels that impairs force production (Findsen et al., 2016), finally eliminating the possibility of movement. To complicate matters further, recovery from chill coma probably involves physiological actions of different organs from those that caused the coma in the first place (MacMillan et al., 2012, 2014). Thus, what appear to be conceptually equivalent measures of thermal limits (impairment in the ability to move or its subsequent recovery) may in fact be entirely physiologically distinct.

If upper and lower thermal limits can be and are being measured in a variety of ways, even within a single taxon, and a seemingly infinite number of limits to other aspects of an animal's performance exist at less extreme temperatures, it begs the question: what are the 'right' thermal limits to measure? Most focus thus far has been on the mechanisms underlying systemic neuromuscular or

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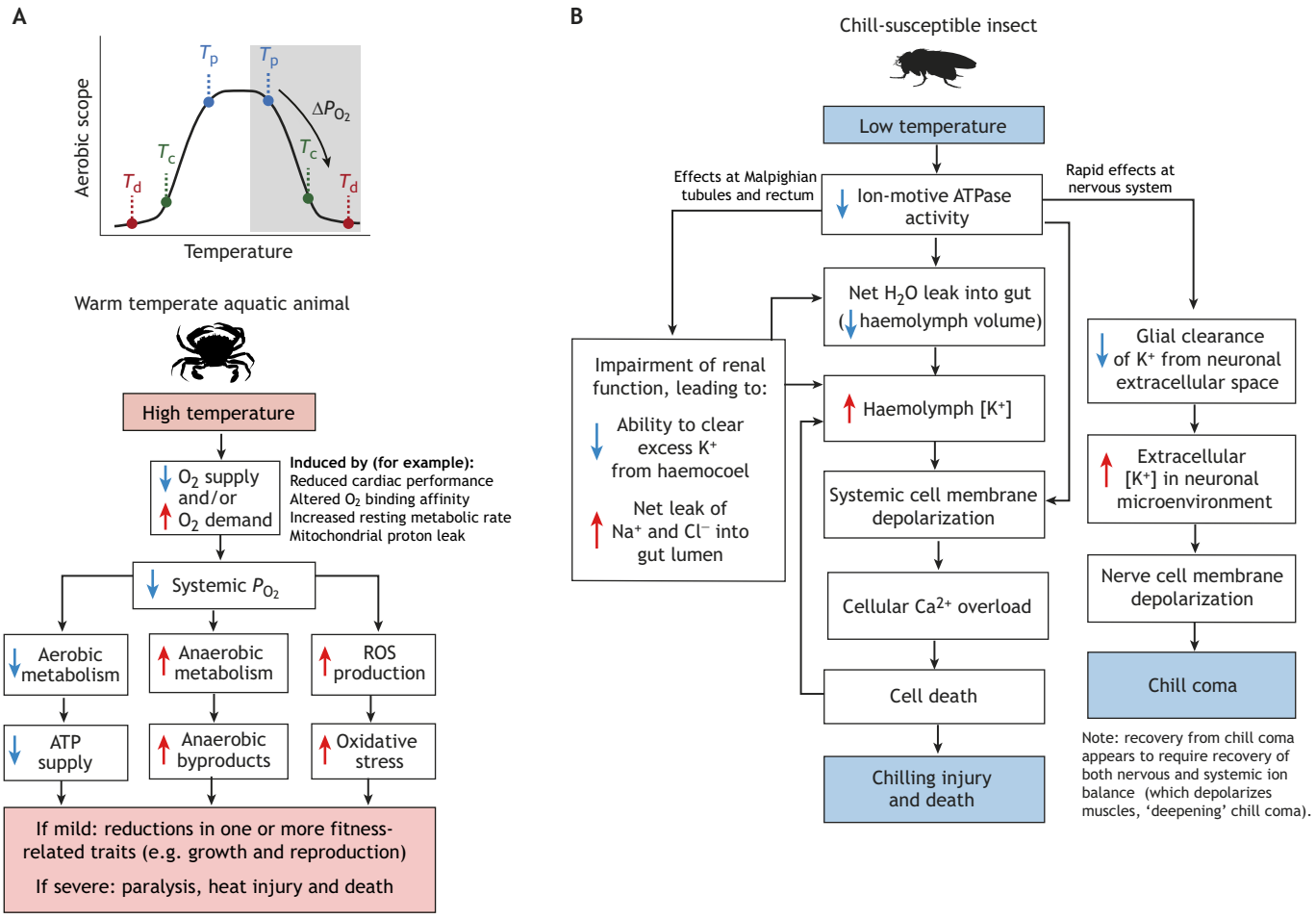

Fig. 1. Two integrative models of the mechanisms setting thermal limits to ectotherm performance. (A) The oxygen and capacity limitation of thermal tolerance (OCLTT) model proposes that limits to animal performance are set by temperature effects on oxygen supply and demand, such that aerobic scope declines at extreme temperatures (inferred from Pörtner, 2012; Pörtner et al., 2017). The grey shaded area highlights temperatures above those that are optimal. T_p represents the pejus temperature (at which tissue hypoxia begins), T_c represents the critical thermal limit and T_d indicates the temperature of death. P_{O_2}, partial pressure of oxygen; ROS, reactive oxygen species. (B) The ionoregulatory collapse model proposes that insects suffer from local and systemic disruptions in ion and water balance that drive chilling injury and the onset of a cold-induced paralytic state (chill coma; model modified from MacMillan et al., 2015b; see also Overgaard and MacMillan, 2017). In both A and B, arrows represent hypothesized cause-and-effect links between physiological phenomena (boxes) hypothesized or observed in organisms at or beyond their thermal limits.

cardiovascular collapse, injury and death (and the mechanistic models that attempt to explain these limits are rooted in studies of these most extreme limits). Which of these limits matter most for determining organismal fitness and performance is likely to be context and taxon specific, but if different traits (i.e. measures of limits) are caused by different underlying physiology, a clear view of which measures of thermal limits are most important for a given species can serve as a catalyst for a mechanistic understanding with predictive power. Answering this question for any species, and putting those answers to good use, requires a collaborative effort between those monitoring animal experiences and behaviour in the field and those focused on manipulating animal conditions in the laboratory.

Mechanisms proposed to set thermal limits

Although thermal physiology has enjoyed a fruitful history, few integrative models have emerged to explain what sets the boundaries of thermal performance. Two such models for thermal limitation are presented in Fig. 1, and are discussed in more detail below.

One conceptual model for understanding thermal limits that has received considerable attention is that of oxygen and capacity limitation of thermal tolerance (OCLTT, see Glossary; Fig. 1A). The OCLTT model emerged from theory on the interactions between temperature and aerobic metabolic supply and demand (Fry and Hart, 1948), and the integration of this concept with thermal performance curves (Huey and Slatkin, 1976). Under this model, thermal limits of all animals (with a particular research focus on upper thermal limits) are set by temperature-induced declines in aerobic scope (see Glossary) due to a mismatch between oxygen supply and demand (Pörtner and Knust, 2007). Declines in aerobic scope at high temperatures have been proposed to be causally related to, for example, the limits of cardiac capacity (Eliason et al., 2011), mitochondrial function (Abele et al., 2002) or oxygen-carrying capacity (Giomi and Pörtner, 2013), in a manner that depends on the environment encountered and the species in question; thus, ventilatory and circulatory systems are quite central to this concept. The downstream effects of this decline in aerobic scope are predicted to be in line with the effects of tissue hypoxia, including decreased ATP supply, increased reliance on anaerobic metabolism (leading to anaerobic by-product accumulation) and oxidative stress (Pörtner et al., 2017). The OCLTT model has rapidly proliferated in the literature and has even been used as the

basis for studies on, and establishment of predictive models for, fish at the ecosystem level (Del Raye and Weng, 2015; Payne et al., 2016). In recent years, however, this framework has also come under increased scrutiny (Clark et al., 2013; Jutfelt et al., 2018), as many authors suggest the framework is overly reliant on correlative evidence (i.e. cause and effect has not been sufficiently demonstrated), does not apply to low temperatures and is limited in its ability to predict temperature-induced declines in fitness in a variety of organisms (Clark et al., 2013; Ern et al., 2015, 2016; Lefevre, 2016; Verberk et al., 2015). A critical point of concern is that OCLTT does not apply at the critical thermal limits, as aerobic scope does not decline at or around these limits in some animals (e.g. Norin et al., 2014), and thus much of the debate over this model has been specifically focused on the central role of aerobic scope in setting thermal limits (Fig. 1A).

A second framework for understanding thermal limits is that of ion and water balance disruption (Overgaard and MacMillan, 2017), which for simplicity I will here call 'ionoregulatory collapse' (see Glossary) (Fig. 1B). This framework emerged out of observations of a loss of extracellular ion balance in insects exposed to low temperatures (Koštál et al., 2004, 2006; MacMillan and Sinclair, 2011b; Rodgers et al., 2010) and integration of these observations with earlier conceptual models of cellular injury in hypoxia and hypothermia (Boutilier, 2001; Hochachka, 1986). Under this framework, neuromuscular failure and systemic injury of insects at low temperatures is suggested to result from a cold-induced failure to maintain ionic and osmotic balance, causing extracellular hyperkalaemia (see Glossary). At a critical temperature, and within seconds to minutes, a surge of K^+ in the extracellular space of the nervous system depolarizes nerve cells, silencing signal transmission (Robertson et al., 2017). Over a longer time scale (minutes to hours), the effects of chilling on gut and Malpighian tubule ion transport rates are thought to drive haemolymph hyperkalaemia (through net K^+ leak and/or haemolymph volume disruption). At the muscles, this time- and temperature-dependent hyperkalaemia causes cell depolarization, triggering Ca^{2+} influx and initiating necrotic and/or apoptotic cell death (Andersen et al., 2017; Bayley et al., 2018; MacMillan et al., 2015a), which probably drives a feed-forward loop of further K^+ release from cells, further cell depolarization and further cell death (MacMillan et al., 2015b). In this framework, a chain of causation is emphasized and has, in some cases, been tested through manipulative experiments (Andersen et al., 2017; Bayley et al., 2018; MacMillan et al., 2015a, 2018). However, the majority of this work has been completed by a limited group of researchers focused mainly on low temperatures, and this model thus remains to be more widely tested.

It has been suggested that thermal limits are set at the highest level of biological organization (Pörtner, 2002), meaning that interactions among organ systems (for animals that have them) will play a significant role in organismal collapse at extreme temperatures (i.e. no organ is an island). The integrative models summarized above for 'complex' animals support this view, as they point to interactions among organs and organ systems as primary modes of failure. Given that temperature exerts its effects on biochemistry, however, any higher-level phenomena must emerge from the direct effects of temperature on subcellular physiology and biochemistry (Pörtner et al., 2017). In turn, the direct causes of organ and system failure must be traceable to cellular and subcellular phenomena, even if those phenomena are occurring in a different organ and thereby creating a higher-level mismatch. It follows that the same would be true for the mechanisms driving changes in thermal limits over evolutionary time or within an individual lifetime (e.g. acclimatization). Thus, to adequately understand thermal limits we need integrative models that extend through all levels of biological organization, and these models need to be grounded in an appreciation of the diversity of environmental and physiological constraints that animals experience.

High and low temperatures: different physics, different effects

The OCLTT and ionoregulatory collapse models emerged from studies on wholly different taxonomic groups living in wholly different environments (aquatic invertebrates and terrestrial insects, respectively), but they also emerged from studies focused primarily on different ends of the thermal performance curve. There is good reason to suspect that high and low temperatures will lead to different forms of systemic failure; temperature is a measure of the average kinetic activity within a system, and too little kinetic activity (cold) and too much kinetic activity (hot) present inherently different challenges on a molecular level. Both extreme heat and extreme cold can disrupt macromolecular flexibility and stability (impacting enzyme reaction rates, for example), and both can denature macromolecular structure (Bowler, 2018; Szyperski et al., 2006). However, the populations of macromolecules (and, by extension, the cells, tissues and/or organs) most sensitive to extreme heat and extreme cold are likely to differ within an organism, and differ depending on the nature of the stress experienced (Clark et al., 2013).

To support the view that cold and heat lead to distinct cascades of failure, I submit an example from insects in relation to ionoregulatory collapse. Repeated observations of disruptions in ion balance in insects at low temperatures naturally led to the hypothesis that exposure of insects to temperatures around the CT_{max} would cause a similar cascade of failure to that seen during cold exposure. In locusts, heat stress causes hyperkalaemia (as is the case in the cold), but at high temperatures this appears to be a secondary consequence of cell death occurring for other, still unknown, reasons (O'Sullivan et al., 2016). This example emphasizes the importance of recognizing the inherent differences of extreme heat and extreme cold, as well as the need to link observed physiological phenomena to their proximate cause (discussed further below). Measuring ion balance alone at high temperatures and relating it to observations of injury, without accounting for the timing of cell death, would have led to erroneous correlative support for the notion that ion balance was causing injury at the whole-animal level. Although hyperkalaemia may contribute to further injury in the heat, it is not the proximate mechanism of cell death in a locust (O'Sullivan et al., 2016). What is directly initiating cell death in insects at high temperatures remains unclear, but what is clear is that progressive hyperkalaemia observed in the cold is a consequence of a biochemical challenge that locusts simply do not face in the same way at their CT_{max}. From a cause-and-effect perspective, cold stress and heat stress are fundamentally different.

Given the different physical nature of high and low temperatures, it is extremely unlikely that any one physiological system is the cause of both upper and lower thermal limits, just as it is unlikely that a single type of thermal limit (upper or lower) is set by the same mechanism under different conditions (see discussion of chill coma above). Thus, any one cascade of mechanisms proposed to explain failure at both high and low temperatures in any animal should be considered improbable, and further effort must be made to identify physiological phenomena occurring at each end of

Table 1. Hypotheses for the mechanisms setting thermal limits in the major organ systems of animals

Organ system	Example hypothesis(es) for cause of reduced fitness and/or systemic failure	Example citations
Circulation	Decline in efficiency of circulation (if circulation is related to oxygen delivery) leading to systemic hypoxaemia (see Glossary), tissue hypoxia and/or hypercapnia (e.g. haemoglobin binding affinity, heart failure).	Barlow et al., 2017; Eliason et al., 2011; Giomi and Pörtner, 2013; Wang et al., 2014
Digestive	Reduced consumption, digestion or assimilation efficiency (e.g. from impairment of epithelial transport), leading to starvation.	Kingsolver and Woods, 1997; McGaw and Whiteley, 2012
Ventilatory	Impaired ventilation frequency, oxygen extraction efficiency and/or CO_2 release at respiratory organs.	Bock et al., 2001; Ern et al., 2014
Musculo-skeletal	Reduced muscle force production, owing to impaired Ca^{2+} release or cell depolarization; failure of synaptic signalling.	Findsen et al., 2016; Klose et al., 2008
Sensory/nervous	Inability to sense and respond to unfavourable or favourable stimuli, maladaptive behaviour or coma.	Abram et al., 2017; Miller and Stillman, 2012; Rodgers et al., 2010
Endocrine	Impaired endocrine signalling, leading to cascading effects on other systems (e.g. through maladaptive signal transduction pathway activation or suppression).	Dupoué et al., 2013; MacMillan et al., 2018
Urinary	Failure of osmoregulatory organs or impaired clearance of ions or nitrogenous waste, leading to systemic loss of osmotic balance or accumulation of toxins.	Yerushalmi et al., 2018
Reproductive	Impaired egg/sperm production leading to a failure to reproduce, egg necrosis leading to infection.	Brante et al., 2003; Donelson et al., 2014; Marshall and Sinclair, 2010
Immune	Decreased immune function leading to an inability to effectively combat pathogens and parasites.	Maniero and Carey, 1997

Example citations provide related ideas or foundational information directly relevant to the example hypotheses provided. Note that subcellular mechanisms of failure may simultaneously affect multiple organ systems, such as altered membrane fluidity (Bowler, 2018) or cytoskeletal disruption (Cottam et al., 2006; Des Marteaux et al., 2018).

the thermal performance curve. As physiological events are identified, they can be carefully incorporated into testable cause-and-effect models of organ or systemic failure specific to a given thermal challenge. Note that specific physiological phenomena are still likely to occur at both ends of the curve (e.g. hyperkalaemia occurs at both low and high temperatures in the locust), but the chain of causation under different conditions may be entirely different.

The challenge of determining cause and effect at the point of collapse

Conceptual models (like those presented in Fig. 1) are not, per se, hypotheses, but can instead be considered as a network of conceptually linked hypotheses (each box and each arrow in Fig. 1 is a hypothesis). Viewed from this perspective, such models can provide a skeleton on which to connect new observations, develop new avenues of research or hang evidence of support or opposition for a single mechanistic link or chain of causation.

If thermal physiology is to provide useful information to conservation biologists and policy makers, physiological models of thermal limits must hold predictive power. Any predictive power thermal physiology can provide relies on a clear understanding of the precise causes of an impairment in animal performance, but how do we accurately and efficiently disentangle cause and effect? With multiple interacting levels of biological organization within an animal, the most plausible expectation is that disruption in any one physiological system will subsequently disrupt all others. If this is the case, our task is not to identify everything that does and does not fail, but to identify what failure causes the sublethal or lethal limit in question. From this view, what matters is: (1) what system failure is coincident with the limit under study and is the order of system failure always the same, and (2) does inducing or preventing failure of a given system induce or prevent failure identified at the organismal level (is failure of the system necessary or sufficient to cause organismal failure or impact performance/fitness). If some or all systems face collapse, correlative evidence for the importance of a particular trait is weak evidence at best. Thus, at the organ system level, determining the causes of failure requires experiments that manipulate the function of the system in question while observing the effects *in vitro*, and reconciling these observations with parallel studies at the whole-organism level (e.g. MacMillan et al., 2018). When strong evidence for a relationship is found in this manner, molecular biology and genetics offer powerful tools for testing cause and effect (i.e. manipulative experiments involving changes in gene expression, cell signalling, post-translational modification, etc.) that can and should be put to use before any model is considered well supported.

A systematic approach to understanding thermal limits

It has been over 50 years since John R. Platt described the power of strong inference (Platt, 1964). Put simply, the strong inference method is a systematic approach to research where multiple competing hypotheses are simultaneously tested via a carefully constructed experiment, such that one or some are supported and others are excluded. Platt (1964) argued that this was the most efficient form of scientific progress and credited this method for

Box 1. A systematic approach to studying thermal limits

Asking the following series of questions can help to rapidly uncover the mechanisms that set a thermal limit of interest. This approach can create the foundational knowledge needed for a deep understanding of the mechanisms determining variation in thermal tolerance among individuals, populations or species.

1. What trait (i.e. thermal limit) is important to our understanding or to the ecology of the animal in its environment?
2. Which system(s) is (are) failing most dramatically under the conditions of this 'important' thermal limit?
3. Within that system, which forms of failure are occurring and which are not? Which markers of failure are predictable and repeatable? (Note: this is how many researchers have tested for a decline in aerobic scope near thermal limits – a prediction of OCLTT).
4. Does a given form of failure arise from or cause other observed forms of failure within or beyond the given organ system (i.e. can we establish causal relationships, considering the organism as a whole)?
5. What (if anything) can be done to alleviate these effects in an experimental context? Where are there nodes of physiological intervention?

rapid advancements in the fields of particle physics and molecular biology at that time. Despite wide recognition for the usefulness of this approach, the majority of research we conduct in thermal biology ignores this method and instead focuses on supporting or refuting a singular hypothesis (e.g. a single box or arrow in Fig. 1). The result of this approach can be significantly slower progress of a field toward a goal of predictive understanding.

By building integrative models with a variety of alternative routes to organismal failure, and by testing those models through a strong inference approach, we can embrace the true complexity and diversity of animal physiology that many lifetimes of research in comparative physiology have revealed, and we can more rapidly advance our understanding of animal thermal limits. Models that emphasize cause and effect, by their very nature, provide these multiple interconnected and competing hypotheses for failure, and their use would help to avoid frustrating debate over a singular hypothesis. Such models can also serve as a base from which to identify and explain the inevitable taxa that do not 'follow the rules', but that may be of critical environmental or economic importance. By identifying and incorporating these species, and interpreting differences in light of their life history, we can better understand the strengths and weaknesses of our models and incorporate and test newly suggested cause-and-effect relationships, thereby improving predictive power.

Some will argue that the precise problem with understanding thermal limits is that we do not have enough competing hypotheses for why organismal fitness declines at low and high temperatures (and thus how can we possibly integrate imaginary hypotheses?). Playing devil's advocate, I would argue that the number of potential causes of organismal failure is equal to the number of thermally sensitive molecular interactions occurring in an animal, and that the most parsimonious expectation is that many forms of systemic collapse are occurring simultaneously (and interacting) in an animal facing an unfavourable thermal environment. This concept is similar to (although perhaps more intimidating than) the multiple performances–multiple optima (MPMO) hypothesis (Clark et al., 2013; see Glossary). The models presented in Fig. 1 each already incorporate multiple organ systems, but a great number of hypotheses for organismal failure remain insufficiently explored. As a start, we should perhaps consider identifying which organ systems are most prone to failure in our favourite species, clade or environment, and from there identify the causes of failure in each of those systems (see Table 1 for examples). In taking this approach, our challenge is not to identify a singular cause of failure but to embrace that many things are probably failing in concert and to systematically probe a series of interrelated and interdependent questions on what limits should be measured, what systems drive those limits and whether experimentally manipulating that system can alter the limit in question (Box 1).

This integrative and systematic approach would allow us to focus research effort on the traits demonstrated to be most ecologically relevant, and it could rapidly lead to mechanistic models that will gracefully evolve along with new evidence. At the least, the existing models represent plausible hypotheses for systemic collapse in some animals and under some conditions, and can thus serve as a scaffold of hypotheses from which a more thorough understanding can begin to be shaped. Importantly, determining this failure cascade is a necessary step in identifying mechanisms of thermal adaptation, including phenotypic plasticity. Once a series of physiological phenomena are identified, it is conceptually simple to postulate and test how adaptive variation in organ and cell physiology directly influences the performance or fitness outcome.

Conclusions

The complexity of integrative models of thermal limitation will undoubtedly grow in the coming years, as new hypotheses are incorporated and tested and as technological advances allow for the simultaneous measurement and analysis of multiple physiological traits in free-living animals (Chmura et al., 2018). In time, I expect we will reach holistic and highly predictive 'physiological failure networks' (akin to physiological regulatory networks; Cohen et al., 2012) that can explain thermal limitation. Like regulatory networks, some nodes (mechanisms) and pathways (cause-and-effect relationships) within these physiological failure networks may be more connected, and thus play a more central role in thermal limitation. These 'central' nodes may be the physiological phenomena we have focused on to date (e.g. aerobic scope, hyperkalaemia), or they may not, and being confident about whether or not we are on the right track will require a great deal more research effort. There is one thing we can be sure of at this point: the mechanisms underlying thermal limits are far more complicated than we like to think.

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