Title

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Journal

Integrative and Comparative Biology, 56(1)

ISSN

1540-7063

Authors

Williams, Caroline M
Buckley, Lauren B
Sheldon, Kimberly S
et al.

Publication Date

2016-07-01

DOI

10.1093/icb/icw013

Peer reviewed

OXFORD UNIVERSITY PRESS | Integrative and Comparative Biology

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Journal:	*Integrative and Comparative Biology*
Manuscript ID	ICB-2016-0047.R1
Manuscript Type:	Invited review
Date Submitted by the Author:	23-Mar-2016
Complete List of Authors:	Williams, Caroline; University of California Berkeley, Integrative Biology Buckley, Lauren; University of Washington, Biology Sheldon, Kimberly; University of Wyoming Vickers, Mathew; CNRS Poertner, Hans - O.; Alfred-Wegener-Institute for Polar and Marine Research, Integrative Ecophysiology Dowd, William; Loyola Marymount University, Department of Biology Gunderson, Alex; University of California, Berkeley; San Francisco State University Marshall, Katie ; University of British Columbia, Zoology Stillman, Jonathon; San Francisco State University; University of California Berkeley
Keywords:	thermal performance curve, acclimatization, plasticity, sub-lethal, damage

Biological impacts of thermal extremes: mechanisms and costs of functional responses matter

Caroline M. Williams[1], Lauren B. Buckley[2], Kimberly S. Sheldon[3], Mathew Vickers[4], Hans-Otto Pörtner[5], W. Wesley Dowd[6], Alex R. Gunderson[1,8], Katie E. Marshall[7], Jonathon H. Stillman[1,8]

[1]University of California, Berkeley, California, USA
[2]University of Washington, Seattle, Washington, USA
[3]University of Wyoming, Laramie, Wyoming, USA
[4]Station d'Ecologie Théorique et Expérimentale, UMR 5321, CNRS 2 route du CNRS, 09200 Moulis, France
[5]Alfred Wegener Institute, Helmholtz Center for Marine and Polar Research, Bremerhaven, Germany
[6]Loyola Marymount University, Los Angeles, California, USA
[7]University of British Columbia, Vancouver, British Columbia, Canada
[8]San Francisco State University, Tiburon, California

Running title: Biological impacts of thermal extremes
Words in text: 5313 (not including refs)

Abstract

Thermal performance curves enable physiological constraints to be incorporated in predictions of biological responses to shifts in mean temperature. But do thermal performance curves adequately capture the biological impacts of thermal extremes? Organisms incur physiological damage during exposure to extremes, and also mount active compensatory responses leading to acclimatization, both of which alter thermal performance curves and determine the impact that current and future extremes have on organismal performance and fitness. Thus, these sub-lethal responses to extreme temperatures potentially shape evolution of thermal performance curves. We applied a quantitative genetic model and found that beneficial acclimatization and cumulative damage alter the extent to which thermal performance curves evolve in response to thermal extremes. The impacts of extremes on the evolution of thermal performance curves are reduced if extremes cause substantial mortality or otherwise reduce fitness differences among individuals. Further empirical research will be required to understand how responses to extremes aggregate through time and vary across life stages and processes. Such research will enable incorporating passive and active responses to sub-lethal stress when predicting the impacts of thermal extremes.

Key words: thermal performance curve, acclimatization, plasticity, sub-lethal, damage, heat, cold

Introduction

Relationships describing the temperature dependence of physiological performance and, ultimately, of fitness are a critical component of predicting the responses of ectotherms to climate change (Deutsch and others 2008; Huey and Berrigan 2001; Vasseur and others 2014). However, such thermal performance curves (TPCs) are generally constructed under constant environmental conditions in the laboratory and, therefore, provide little insight into the biological consequences of transient exposure to extreme temperatures. In a growing number of examples, the role of episodic exposure to extreme temperatures rivals that of mean temperatures in driving organismal responses (Clusella-Trullas and others 2011; Denny and Dowd 2012; Garland and others 2015; Hoffmann 2010; Marshall and Sinclair 2015; Paganini and others 2014). This conclusion is supported by examples from the field and the laboratory, and across terrestrial, aquatic, and intertidal systems.

Thermal extremes clearly shape the evolution of some components of organismal thermal responses, with impacts that reverberate throughout the communities and ecosystems in which individual organisms operate. Organismal responses to extreme temperatures often involve sub-lethal thresholds, such as constraints on aerobic metabolism and energy budget (see below), the induction of heat shock protein synthesis, or acute losses of equilibrium at critical thermal maxima/minima (Hochachka and Somero 2002; Pörtner 2001; Somero 2010). Crossing these thresholds induces carryover effects resulting from functional constraints, damage accumulation or acclimatization, and the magnitude of these carryover effects will depend on exposure number, duration and intensity, and the interval time between events (Somero 2010). These carryover effects include passive accumulation of damage and loss of performance (e.g., resulting from oxidative stress), and also active acclimatization responses. Carryover effects of sub-lethal exposure to thermal extremes impact responses to future extremes, and so incorporating carryover effects into forecasts of responses to climate change is likely to improve predictive power, particularly in systems where exposure to extreme temperatures is driving organismal responses to climate change (Gunderson and others 2016; Woodin and others 2013).

Incorporating carryover effects of extremes into forecasts of future biological responses to climate change requires a better mechanistic understanding of underlying biochemical and physiological phenomena induced by extreme events. These requirements are two-fold. First, it is important to clarify the relevant sub-lethal limits that influence physiological responses to extreme temperatures, including whether those limits are generalizable across taxa, habitat types, and types of extremes (e.g., warm vs. cold, single warm/cold days vs. anomalously warm/cool years). Second, biologists must better quantify the physiological costs of sub-lethal extreme exposures (Denny and Dowd 2012; Dowd and others 2015; Paganini and others 2014), by integrating functional genomic, biochemical, and physiological processes that coordinate function at higher levels of organization (Stillman and Tagmount 2009). Finally, mechanistic linkages between organismal and higher-order ecological and evolutionary responses are needed for a predictive understanding of how ongoing climate change will reconfigure biological diversity (Pörtner and others 2006).

The central goal of this review is to promote mechanistic exploration of sub-lethal physiological consequences of exposure to temperature extremes, particularly of the nature and

magnitude of carryover effects and their implications for predicting the impacts of climate change. Vulnerability to climate change depends on the degree of *exposure* (set by extrinsic factors) and physiological *sensitivity* (set by intrinsic factors) (Williams and others 2008). We identify when and where extreme temperature exposure is likely to be particularly important. We review the functional responses setting sensitivity to extreme temperatures, with particular reference to active and passive processes driving carryover effects. We use an evolutionary model to investigate how these carryover effects might drive the evolution of TPCs in response to thermal extremes. We conclude with an analysis of the effects of thermal extremes on ecological and evolutionary patterns.

Where and when are organisms exposed to extreme body temperatures?

Statistically and meteorologically, an extreme temperature event is defined as a rare event within the statistical reference distribution of events at a particular place (Houghton and others 2001). This remains a useful starting place to understand large-scale patterns of potential exposure to environmental extremes (Dillon et al. this issue), but realized exposures are modified by the interactions among animal behavior, biophysical processes, and habitat heterogeneity (Huey and others 2012; Kearney 2012). Some animals can behaviorally modify their exposure to extremes through the selection of thermally favorable microclimates (behavioral thermoregulation), by escaping in space (migration), or by being active only during certain times of the year (hibernation and/or quiescence, e.g. dormancy). Thus, quiescence or mobility can reduce the importance of extremes relative to means. Increased habitat heterogeneity likewise reduces the relative importance of extremes: not all individuals will be exposed to all extremes (Denny and others 2011). Habitat thermal heterogeneity varies predictably with habitat type, with (for example) heterogeneity generally higher on land versus in water, in intertidal relative to subtidal aquatic systems, or in mesic forest versus xeric scrub (Gunderson and Leal 2012; Suggitt and others 2011; Woods and others 2015).

Environmental extremes also vary in predictable ways with geography. Using global estimates of air and ocean temperatures as a reference, there are clear biogeographic patterns in the incidence and magnitude of extremes. Latitudinal or altitudinal clines in air temperature extremes are less smooth, and often shallower, than clines in mean temperatures (Dillon et al. *this issue*). Minimum and maximum air and water temperatures both decrease with increasing latitude and altitude, potentially leading to decreased exposures to extreme heat and increased exposures to extreme cold with increasing latitude and altitude (Sunday and others 2011). Clines in maximum and minimum temperatures have different slopes, such that exposure to cold extremes changes far more with latitude and altitude than exposure to heat extremes. Perhaps the most pronounced biogeographic distinction impacting the magnitude and incidence of extremes is that between water and land. Thermal capacity of air is low relative to water, thus temperature changes occur more rapidly in air, meaning that on average terrestrial organisms are exposed to greater magnitudes of extreme temperatures (Sunday and others 2011).

To determine impacts of extremes on organisms, environmental temperatures must be mapped to body temperatures (also called operative temperatures; Bakken and Angilletta 2014). In some cases, using operative temperatures modifies or even reverses geographic patterns in exposure to extremes: for example, small ectotherms across latitude have an equal chance of being exposed to extreme heat when body temperatures are explicitly considered

(Sunday and others 2014). Additionally, synergistic interactions among stressors mean that extreme ecological impacts can arise from a combination of individual factors that are not extreme individually (Denny and others 2009). Thus, translating physical (e.g. climate) variables to characteristics relevant to the organism, such as body temperature, is a necessary step toward evaluating the effects of interacting stressors on organisms.

Extreme thermal events can occur on a range of timescales relevant to organisms, including daily cycling, multi-day events (e.g., weather fronts on land, extreme low tide series in the intertidal zone), and seasonal, annual, and multi-annual cycles (e.g. North Atlantic Oscillation, ENSO and PDO). The relative incidence of extremes at each of these timescales changes according to biogeography; for example the magnitude of daily relative to seasonal air temperature variation declines from the tropics to the poles (Wang and Dillon 2014).

Regardless of where or when they occur, extreme temperatures cause organismal impacts because they may push organisms outside critical limits for performing vital functions. Thus, environmental extremes must be evaluated with respect to thermal tolerances of organisms to infer the functional consequences of extreme temperature exposure in the field.

Functional responses to extreme temperatures

The links between mechanistic, physiological constraints and organisms' sensitivity to extreme temperatures are generally well-established (Huey and others 2012). Beyond absolute upper and lower lethal limits, extreme temperatures rapidly induce mortality due to catastrophic cold or heat shock. This mortality results from protein denaturation, membrane phase transitions, loss of transmembrane gradients, or, in the case of extreme cold temperatures, uncontrolled freezing of intra- and extracellular water (Hochachka and Somero 2002). Within the temperature range over which an organism can survive are various thermal thresholds that delineate the onset of sub-lethal effects (Fig. 1; Huey and Kingsolver 1989).

Thermal thresholds, such as those shown in Fig. 1, are not static and can be modified by both passive and active processes occurring during and after exposure to extreme temperatures. Passive processes include cumulative damage incurred or negative energy balance induced by time spent outside critical limits. Limitation in the capacity of oxygen supply to meet demand is a primary mechanism setting responses to extreme temperatures for water-breathers, given the low solubility of oxygen in aquatic environments (Pörtner, 2010). At warmer temperatures, falling oxygen solubility in water is compensated for by increasing oxygen diffusivity (necessitating concepts such as the oxygen supply index, OSI) (Verberk and others 2011), highlighting the role of thermal constraints on ventilatory and circulatory capacity for meeting oxygen demand (Pörtner 2010). Despite increased OSI at warmer temperatures, temperatures outside critical limits for organismal function impose systemic limitation in oxygen supply relative to demand, which in turn leads to hypoxemia and imposes stress at the molecular and biochemical levels (Pörtner 2010). Thermal extremes reduce mitochondrial coupling due to changes in membrane fluidity, increasing oxidative stress. Hypoxemia leads to the onset of anaerobic metabolism lowering metabolic efficiency (Heise and others 2006; Sommer and others 1997; Zielinski and Pörtner 1996). The importance of systemic oxygen limitation in setting thermal limits is poorly established in terrestrial environments (Smith and others 2015; Verberk and others 2016). Oxygen concentrations about 30-fold higher in air than in water likely have alleviated thermal constraints on whole organism oxygen supply (Giomi and

others 2014). At the biochemical level, extreme hot and cold temperatures can shift protein structure to conformations that are less binding-competent, leading to a decline in the efficiency of energy production (Hochachka and Somero 2002). Severe thermal extremes can cause conformational shifts that expose hydrophobic core regions of proteins, leading to damaging aggregation; such proteins are typically degraded through ubiquitin-mediated proteolytic processes, causing a large energetic loss (Hochachka and Somero 2002).

Some functional consequences are specific to the nature of the extreme. During hot extremes, the efficiency of mitochondrial energy production declines due to progressive uncoupling (Leary and others 2003). A decline in mitochondrial coupling increases free radical production and augments the oxidative stress imposed by hypoxemia (Tomanek 2015). Cold temperature extremes may cause freezing of the body water. Freezing usually represents a lethal limit, but some organisms, including many insects, molluscs, and amphibians, can survive freezing of body water. For these animals, freezing represents a sub-lethal stress, as energetic costs of freezing can induce negative energy balance (Sinclair and others 2013b). Alternatively, freezing may yield energetic benefits by reducing metabolic costs while frozen (Irwin and Lee 2003). The relative costs and benefits of freezing depend on the number and duration of freezing events. Fewer long events are favorable, due to reduced costs of initiating freezing and increased metabolic savings while frozen (Marshall and Sinclair 2012). Costs are also modified by temperatures experienced while frozen - colder is better, provided animals remain above their lower lethal temperature (Voituron and others 2002).

In summary, passive effects of thermal extremes include a loss of metabolic, ionic and osmotic homeostasis, which progressively worsen during exposure to extreme temperatures. The damage accumulated and the energy lost during exposure to thermal extremes makes survival beyond these sub-lethal limits dependent upon time and temperature (i.e., intensity) of exposure (Pörtner 2010; Woodin and others 2013). Barring sufficient physiological intervention, these passive processes might be expected to severely constrain subsequent thermal performance, particularly if the consequences carry over between extreme events. The costs of repairing damage and restoring homeostasis may further impinge upon energy budgets, effectively narrowing the thermal window for higher-level functions such as growth and reproduction (Pörtner 2010; Sokolova and others 2012).

To counter these passive consequences, organisms invoke active compensatory responses (plasticity or acclimatization) when faced with thermal extremes. One mechanism is metabolic dormancy, or quiescence, such as in developmental diapause when reduced metabolic demands allow for far greater tolerance levels (Podrabsky and Hand 2015). Under extreme environmental conditions, organisms also employ a conserved set of molecular responses termed the Cellular Stress Response (CSR) (Kültz 2005). Many CSR mechanisms are involved in well-described functions for maintenance of cellular homeostasis, whereas other CSR elements require further analysis to elucidate their functional significance (Kultz 2005). Well-understood CSR mechanisms include responses to protein damage, which is countered by increased expression and activation of molecular chaperones, predominantly heat shock proteins (HSPs) (Feder and Hofmann 1999; Rinehart and others 2007; Tomanek 2015). Membrane phase transitions are countered by changing the composition of lipid membranes (Cossins and Macdonald ; Hazel 1995), sometimes rapidly (Williams and Somero 1996). Increases in oxidative stress are generally countered by CSR up-regulation of antioxidant

defenses (Pörtner 2010), but this is not always sufficient to fully counter the negative impacts of temperature extremes (Abele and others 2002; Jimenez and others in press). When cellular damage exceeds thresholds, apoptosis programs are triggered (Yao and Somero 2012; Yi and others 2007) and irreversibly damaged proteins are targeted for destruction via the ubiquitin-proteasome pathway. Responses to thermal extremes with less well-characterized functions include up-regulation of genes involved in immune responses (Stillman and Tagmount 2009; Zhang and others 2011). It is still unclear whether up-regulation of immune response genes results from increased probability of infection or damage, or shared regulatory mechanisms due to a generalized cellular stress response (Kultz 2005; Sinclair and others 2013a; Todgham and others 2005).

The downstream effects of active responses to thermal extremesthe CSR can include shifts in critical thermal limits, causing the TPC to change over time (Ronges and others 2012; Stillman and Tagmount 2009) and/or in levels of defense against subsequent events. Consequently, the position of critical and lethal limits, and hence the characteristics of the TPC, can change over time. Plastic changes in TPCs can be induced at many points during the life cycle of an organism, and their effects can persist for varying amounts of time. *Acclimatization* (or *acclimation* if it occurs in the laboratory) is a reversible physiological response to temperature change that happens on the order of minutes to days (Angilletta Jr 2009; Brattstrom and Lawrence 1962; Maness and Hutchison 1980). In contrast, *transgenerational* plasticity occurs when temperatures experienced by parents influence TPCs of offspring (Donelson and others 2012; Salinas and Munch 2012) and *developmental plasticity* occurs when temperatures experienced during development influence the TPCs of adults (Gray 2013; Piyaphongkul and others 2014; Scott and Johnston 2012). Thus, TPCs can potentially change on the order of minutes to years due to the various forms of plasticityver the course of the day (hardening or stress responses), over longer periods or ontogeny (acclimatization, whether beneficial or not), and as a result of evolution of plasticity (Angilletta Jr 2009; Kingsolver and others 2011; Schulte and others 2011). In the next section we explore potential interactions between acclimatization, which is probably the most widely studied and best understood form of TPC plasticity, and the evolution of TPCs. among these effects.

Both active (i.e. acclimatization) and passive (i.e. damage or loss of performance) responses to thermal extremes can be costly (Krebs and Feder 1998; Krebs and Loeschcke 1994), producing negative carryover effects of thermal extremes. The magnitude and persistence of those costs, however, have rarely been quantified in sufficient detail to permit their use in evolutionary models (Somero 2002). On the other hand, active acclimatization responses can produce beneficial carryover effects, mitigating impacts of future extremes. Here, we examine the relative importance of costly versus beneficial carryover effects in driving the evolution of thermal performance curves.

How do physiological responses to extremes drive the evolution of TPCs?

A quantitative genetic model [Buckley and Huey this issue] suggests that thermal extremes drive the evolution of TPCs more when they cause mortality than when they have only acute impacts on performance. We extend this consideration of the evolutionary impacts of extreme events in light of the physiological mechanisms presented here. We focus on carryover

effects in response to repeated exposure to warm extremes. We consider a TPC that directly determines fecundity via resource acquisition.

Our model follows the methods outlined in Buckley and Huey [this issue]. We use a beta curve to model the evolution of TPC minima and breadth (Supplementary methods). We assume genetic variances (heritabilities) of 0.7 and covariances of -0.1. We assumed the area under the TPC is fixed and, thus, we omit "hotter is better" (Angilletta and others 2010). We derived our temperature data from the Melbourne, Australia station (#086071) of the Australian Climate Observations Reference Network (http://www.bom.gov.au/climate/change/acorn-sat/). We estimated a kernel density function for daily maximum temperatures spanning the years 1910 to 2014 and generated a time series of 300 daily temperatures from the distribution for each generation (functions kde and rkde from the R library ks). We omitted seasonality and examined 200 individuals with traits generated from a normal distribution with a fixed variance and evolving mean for each of 200 generations (a sufficient number of generations to reach equilibrium). We used daily maximum temperature because we were particularly interested in evolution in response to extremes, but note that finer resolution temperature data would more realistically model the magnitude of selection. We introduced microclimate heterogeneity and assumed the organism was able to behaviorally thermoregulate ~~as described in Buckley and Huey [this issue]~~ (Supplementary methods).

We examined two primary scenarios in which thermal extremes result in either (1) permanent loss of performance (e.g., damage to metabolic machinery) or (2) death. For each scenario and generation, we considered three plausible physiological responses: (a) the impact of each extreme was independent of incidence (i.e., no carryover effects); (b) the impact declined with each subsequent extreme (i.e., beneficial acclimatization); and (c) the impact intensified with each subsequent extreme (i.e., cumulative damage). As heuristic examples, and in light of the scarcity of data quantifying the costs and benefits of cumulative damage and beneficial acclimatization, respectively, we made some simplifying assumptions regarding these parameters. For the first scenario, under permanent loss of performance, we assumed that performance was permanently reduced in an additive fashion by 2% with each extreme temperature (warmer than CT_{max}) encountered. For the remaining physiological responses, we assumed that the percent performance lost was increased (cumulative damage) or decreased (beneficial acclimatization) by 2% with each subsequent extreme temperature (i.e., we multiply performance lost by a factor describing carryover effects). For the second scenario, in which extremes influence only survival, we assumed that survival declines exponentially from 1 at CT_{max} to 0 at 60°C and that there is no effect of exposure time on survival. We assumed that survival rate increased (beneficial acclimatization) or decreased (cumulative damage) by 2% with each subsequent extreme temperature.

Beneficial acclimatization, or cumulative damage in response to thermal extremes, dramatically alters selection on TPCs (Fig. 2). We find that selection can be relaxed if acclimatization reduces differences in relative fitness between individuals with differing critical thermal limits. For the first scenario of permanent loss of performance, cumulative damage selects for greater critical thermal limits than impacts that are non-cumulative (no carryover effects). In contrast, beneficial acclimatization leads to a decrease in critical thermal limits by decreasing selection. For the second scenario, where extremes cause mortality, beneficial acclimatization reduces selection for elevated thermal limits only slightly relative to the case of

no carryover effects. This occurs because dead individuals do not acclimatize. Interestingly, evolution assuming cumulative damage results in lesser thermal tolerance than no carryover effects or beneficial acclimatization, because the performance loss is sufficiently severe to minimize fitness differences and reduce the efficiency of selection. Thermal extremes that kill off most individuals have little impact on the evolution of TPCs, and the TPC largely reflects selection to perform in more average conditions.

Our analyses highlight that carryover effects such as cumulative damage and beneficial acclimatization can alter TPC evolution. The magnitude of carryover effects influences TPC evolution (Fig. S1), indicating that both cumulative damage and beneficial acclimatization are ripe for more detailed physiological investigation. The onset of cumulative damage is likely to be more complex than we assume. For example, cumulative damage should reflect the duration and intensity of extremes, and beneficial acclimatization likely ceases and cumulative damage initiates once the incidence of stress crosses some threshold. An extension of the model to increase realism would be to include mortality and acclimatization / damage simultaneously, since there will always be some hard limits to absolute tolerance that causes mortality (Denny and Dowd 2012).

Selection on the physiological mechanisms outlined above will also depend on factors including genetic correlations and constraints. Trade-offs between basal and inducible tolerance may cause acclimatization capacity to decline as organisms evolve heat tolerance (Stillman 2003). Organisms adapted to variable environments may have high baseline resistance to extremes, but may be less able to mount responses to rare, exceptional extremes. For example, organisms from variable environments that constitutively express high levels of heat shock proteins can have less capacity to induce expression of additional proteins (Stillman and Tagmount 2009), but this tradeoff is far from universal (Calosi and others 2008; Gunderson and Stillman 2015). In addition, the degree to which thermal exposure effects carry over across different life stages is an open question. Some studies suggest that carry over effects may be minimal, and that thermal performance across life stages may be relatively decoupled (Kingsolver and others 2011; Potter and others 2011). We do not yet know enough about cross-life stage correlations in TPCs to make any general predictions on how such processes will modify evolutionary responses to thermal extremes, but this is an interesting area for future research.

How do extremes impact ecology and evolution?

Evolutionary tradeoffs related to TPCs can govern responses to extreme temperatures (Kingsolver 2009). The first evolutionary pattern -"hotter is better"- results from higher performance at warmer temperatures due to release from biochemical and physiological constraints (Angilletta and others 2010). "Hotter is better" could shift thermal tolerance to warmer temperatures and make organisms better able to cope with extremes. Additionally, more energetically costly life cycles are possible at high temperatures, which may enable organisms to cope with the energetic costs of warm (but not cold) extremes. Genetic correlations may, however, result in selection for higher thermal optima, thus reducing thermal tolerance breadth.

A second evolutionary tradeoff related to TPCs is between specialists and generalists. Whether temperature variation will select for broader thermal tolerances depends on the

timescale of variation relative to generation time. High within-generation variation can slow selection, but can ultimately result in thermal specialization; high between-generation variation maintains performance breadth (Gilchrist 1995). Diurnally and seasonally constant tropical climates select for specialized thermal tolerances such that even small temperature anomalies can be stressful (Deutsch and others 2008; Janzen 1967; Sheldon and Tewksbury 2014).

A third evolutionary tradeoff related to TPCs is between faster and slower life cycles. Intermittent extremes may favor the evolution of a rapid life cycle to avoid extremes (Stearns 1976). This would allow many generations with high population growth to buffer occasional generations facing reduced population growth due to extremes. Alternatively, physiological mechanisms of coping with extremes (e.g., hardening response or expression of HSPs) may be energetically costly and thus slow life cycles. Thermal extremes may also determine the evolution of voltinism (Nilsson-Örtman and others 2012). Organisms may synchronize their life cycle with seasonal or otherwise periodic extreme events (e.g., summer dormancy to avoid desiccation or winter diapause). This synchronization requires the evolution of a phenological response and can slow the life cycle. Overall, life cycles will evolve to correspond to timescales of variation.

Gene flow among populations distributed along climatic gradients also influences sensitivity to thermal extremes. Selection to tolerate extremes can be distinct from selection on mean thermal tolerance such that gene swamping from the center to edge of a distribution may keep edge populations vulnerable to extremes (Kirkpatrick and Barton 1997; Paul and others 2011). Stressful, extreme temperatures at a species' range edge reduce demographic fitness parameters (Crozier 2004; Descamps and others 2015; Hassall and others 2006; Sanz 1997; Sexton and others 1992), and in some cases set and maintain range edges. Consequently, ranges often shift in punctuated steps coincident with extremes rather than gradually in response to mean climate changes (Harley and Paine 2009; Wethey and others 2011). Thresholds, where sub-lethal constraints take effect, correlate with biogeographical limits (Deutsch and others 2015; Frederich and Portner 2000; Root 1988).

The impacts of extremes can be intensified by shifts in species interactions. Warm or cold spells can lead to phenological mismatches with strong, negative impacts on fitness when key food resources or primary pollinators are missing (Miller-Rushing and others 2010; Reed and others 2013). Extreme temperatures can also alter species' interactions through shifts in physiological performance due, for instance, to inducing energetically costly protection against extremes (Urban and others 2012). Increased incidences of extreme temperatures with resulting strong selection on thermal tolerances can reduce species diversity and impact community functioning (McClanahan and Maina 2003; Pincebourde and others 2012). Performance shifts associated with increases in temperature variability have also been shown to alter host-parasite interactions, including sensitivity to disease and host immunity (Murdock and others 2012). Extreme temperatures can also alter ecosystem scale processes. For example, increased exposure to extreme low temperatures can alter physiological functioning and increase mortality of insect pests with consequences for forest health (Marshall and Sinclair 2015).

Conclusions and future directions

The potential for thermal extremes to drive the evolution of organismal physiology by causing mortality is well documented (Gilchrist 1995; Levins 1968). Less appreciated are the

many sub-lethal stress responses that are the focus of our review. Organismal response to sub-lethal stresses that differentiate individual fitness and determine survival can drive the evolution of TPCs, as we see from our model. Sub-lethal thermal stress affects fitness via mechanisms including reduced fertility or reproductive output, a reduction in offspring performance or development, and energetic costs of hardening or repair. Do these responses aggregate in a manner such that a TPC (usually quantified based on a single performance metric) is a reasonable approximation of the temperature dependence of organismal performance and fitness (Kingsolver and Woods 2016)? Or do thresholds and other non-linear responses aggregate in a manner such that standard empirical measures of TPCs are inadequate to capture the performance and fitness implications of thermal extremes? To address these questions, we must consider the underlying physiological mechanisms in operation outside the range of optimal temperatures of the TPC, where systemic and biochemical constraints dictate the precipitous fall in performance, and resources must be reallocated to damage control through the production of HSPs or other mechanisms. Our analysis suggests that the manner in which repeated extremes aggregate have important implications for evolution of TPCs in response to extremes. Beneficial acclimatization is only able to lessen thermal stress and reduce fitness differences if individuals are able to survive the initial stress. The accumulation of stress or damage across events can result in mass mortality events, which can weaken directional selection associated with thermal extremes and increase the relative importance of selection to maximize performance at average temperatures. Despite the simplifying assumptions of our model, we illustrate how carry-over effects will complicate predictions of how TPCs will evolve in response to future climates given increases in duration, intensity, or frequency of extreme events (Diffenbaugh and Field 2013).

These findings relate to ongoing discussions of whether plasticity will facilitate or hinder evolution in response to climate change (Hendry 2016; Merilä and Hendry 2014). Beneficial acclimatization lessens selection for elevated thermal tolerance in response to moderate thermal stress. However, when thermal stress becomes sufficiently severe, beneficial acclimatization can enable sufficient levels of survival to allow selection to act on differences among individuals in the ability to survive thermal extremes. However, our analyses vastly simplify the diverse mechanisms of acclimatization. It will thus be difficult to predict whether acclimatization, and plasticity more generally, will facilitate or hinder evolution for particular organisms. Two recent macrophysiological studies that focused on different aspects of TPCs concluded that plasticity cannot fully compensate for rising environmental temperatures (Gunderson and Stillman 2015; Seebacher and others 2015). Thus, the extent of acclimatization may fall in a middle ground where it enables survival and allows selection to act.

Coordinated research initiatives will be required to understand how biochemical and physiological mechanisms aggregate to shape TPCs and the extent to which TPCs are shaped by thermal means versus extremes. Documenting the onset and costs of numerous mechanisms of sub-lethal stress and comparing populations from different environments and individuals from different ontogenetic stages in the same species will be central to this work (Kingsolver and others 2011). TPCs should also characterize multiple aspects of performance (e.g., locomotion, feeding and assimilation, development, reproduction) (Kingsolver and others 2011). Ideally, measures of physiological and biological consequences will be assessed in response to the same thermal stress. Discrepancies in experimental protocols such as exposure

time or ramping rate make comparisons such as those in Figure 1 difficult, even for well-studied species. Further, estimates of performance and fitness are generally based on constant environments. Incorporating fluctuations and realistic temperature variability will enable an understanding of the relative contributions of thermal means and extremes to the evolution of organismal physiology. High levels of temperature variation can expose organisms to heat and cold stress, but conversely can extend the duration of exposure to optimal temperatures before and after the stressful temperatures (Kingsolver *et al.*, 2015; Ma *et al.*, 2015).

Using TPCs to understand organismal responses to thermal extremes requires careful consideration of how physiological responses aggregate over time. The timescales of exposure to temperature may shift what would be considered an "extreme" in so far as physiological responses are concerned. At short timescales (e.g., one solar day), an extreme weather event could result in an extremely hot or cold exposure beyond critical thermal thresholds, with large consequences for physiology and fitness. However, at longer time scales, repeated exposure to lower temperatures could have the same operative effect. One week of exposure to temperatures below the critical threshold could be just as damaging to fitness. Those temperatures, which would have nearly no discernible impact on a daily or weekly time frame, could have damaging fitness consequences if continuous exposure to those temperatures results in a chronic energy imbalance. One possible way to account for the aggregation of stress over time would be to construct performance curves where accumulated exposure to extremes replaces temperature on the x-axis.

Given the challenges of assessing the impacts of extremes, can we identify those cases where predicting climate change responses will require considering thermal extremes? Comparing the magnitude of environmental variation to the temperature range between physiological stress and mortality could provide information about whether organisms are more constrained by means or extremes (Woodin et al., 2013). Cases where organisms are constrained by extremes may require moving beyond TPCs to consider the physiological factors limiting responses to the extreme events (cf. Pörtner 2010).

Even simple models based on TPCs for single performance metrics reveal that extreme temperatures can have dramatic ramifications for the physiology, ecology, and evolution of organisms. Understanding the impacts of thermal extremes on organisms will require quantifying the mechanisms by which organisms respond to sub-lethal thermal stress and sustain passive tolerance over limited time periods (Pörtner 2010). These mechanisms determine how stress accumulates over time for individuals and how the stress responses of individuals aggregate across populations, species, and communities to determine biodiversity and ecosystem-level responses to climate change.

Funding

The symposium was made possible by generous support from the National Science Foundation (IOS-1545787 to A. Woods, M. Dillon and M. Sears), the Company of Biologists, and The Society for Integrative and Comparative Biology. This work was supported by the National Science Foundation [DBI-1349865 to L.B., Postdoctoral Research Fellowship 1306883 to K.S.S., IOS-1256186 to W.W.D. , IOS-1451450 to J.H.S.], LABEX TULIP (ANR-10-LABX-41 to M.V.), the French National Research Agency (ANR) program open call INDHET (to M.V.), a Natural Sciences and Engineering Research Council of Canada Postdoctoral Fellowship (to K.E.M), and the AnaEE-France project (ANR-11-INBS-0001 to M.V.).

Acknowledgements

We thank the organizers of and participants in the SICB Beyond the Mean workshops, particularly A. Woods, M. Dillon, M. Sears, R. Huey and J. Kingsolver, for ideas. We thank Silvain Pincebourde and David Vasseur for comments that improved the manuscript.

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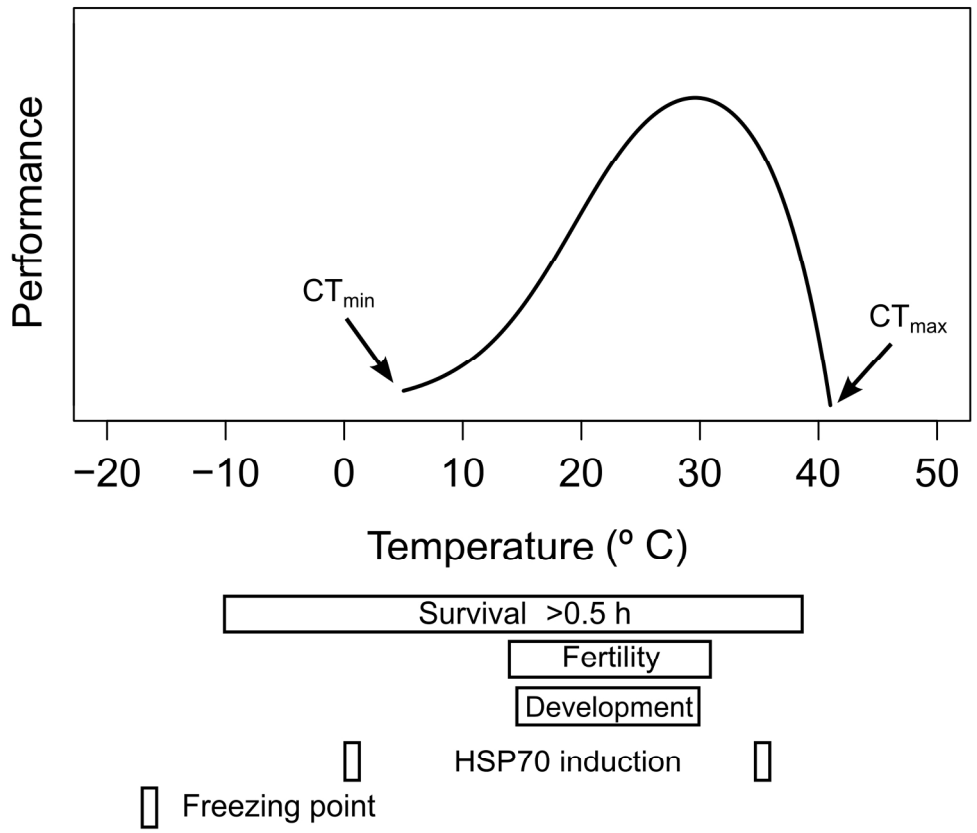
Figure Captions

Figure 1. Thermal performance curve (TPC) for walking speed of *Drosophila melanogaster*, with critical limits for other organismal functions indicated by bars below. The TPC is asymmetric and bounded at the extremes by critical limits, in this case delineating the acute loss of walking ability. Performance generally decreases on either side of a thermal optimum (T_{opt}), with a shallow decrease towards the lower critical limit (CT_{min}) and a steep decline to the upper critical limit (CT_{max}). Outside these critical limits, survival is time and temperature dependent. Between CT_{max} and CT_{min} lie progressively narrower limits for higher-level organismal functions such as development and fertility. At temperatures near CT_{min} and CT_{max}, molecular chaperones such as HSP_{70} are induced to offset temperature effects on macromolecular structure. Our discussion focuses on body temperatures near and beyond these critical limits, at both ends of the thermal window. Note that discrepancies in experimental protocols, such as the time-scale of exposure for measures of motor performance versus those for development, make direct comparisons difficult but still conceptually useful. Data from Gilchrist and others (1997); Czajka and Lee (1990); Stetina and others (2015); Sinclair and others (2007); Kelty and Lee (2001); Siddiqui and Barlow (1972); Klepsatel and others (2013).

Figure 2. Carryover effects such as beneficial acclimatization to thermal stress (dashed lines) and cumulative damage (dotted lines) impact the evolution of thermal performance curves (TPCs). In most cases, thermal extremes drive the evolution of TPCs more strongly when they cause mortality (gray lines) than when they cause sub-lethal performance reductions (i.e., injury; black lines). If cumulative damage intensifies with each incidence of an extreme, evolution selects for less thermal tolerance when extremes cause mortality and greater thermal tolerance when extremes only impact performance. The thick, light grey line depicts the case when the impacts of extremes are restricted to short term performance (i.e., no mortality or lasting performance reductions). The temperature distribution (shown as shaded gray silhouette) is derived from daily maximum temperatures in Melbourne, Australia.



Thermal performance curve (TPC) for walking speed of *Drosophila melanogaster*, with critical limits for other organismal functions indicated by bars below. The TPC is asymmetric and bounded at the extremes by critical limits, in this case delineating the acute loss of walking ability. Performance generally decreases on either side of a thermal optimum (T_{opt}), with a shallow decrease towards the lower critical limit (CTmin) and a steep decline to the upper critical limit (CTmax). Outside these critical limits, survival is time and temperature dependent. Between CTmax and CTmin lie progressively narrower limits for higher-level organismal functions such as development and fertility. At temperatures near CT_{min} and CT_{max}, molecular chaperones such as HSP_{70} are induced to offset temperature effects on macromolecular structure. Our discussion focuses on body temperatures near and beyond these critical limits, at both ends of the thermal window. Note that discrepancies in experimental protocols, such as the time-scale of exposure for measures of motor performance versus those for development, make direct comparisons difficult but still conceptually useful. Data from Gilchrist and others (1997); Czajka and Lee (1990); Stetina and others (2015); Sinclair and others (2007); Kelty and Lee (2001); Siddiqui and Barlow (1972); Klepsatel and others (2013).
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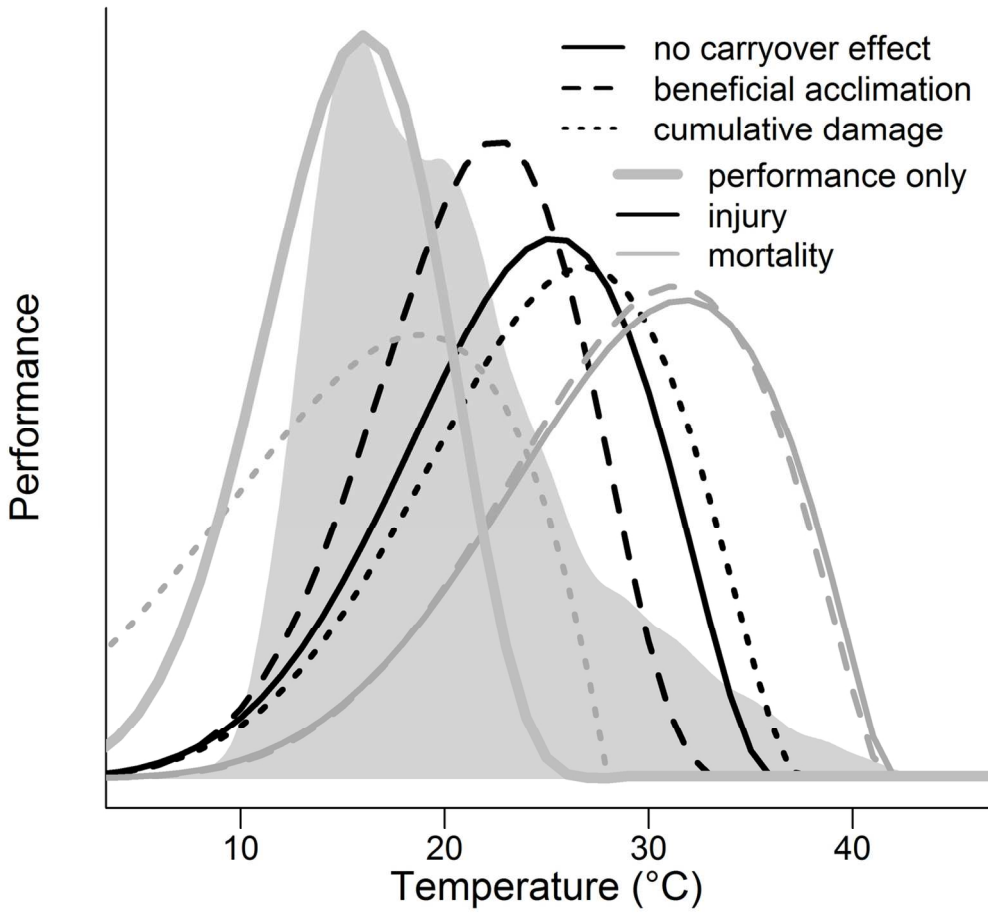

Carryover effects such as beneficial acclimatization to thermal stress (dashed lines) and cumulative damage (dotted lines) impact the evolution of thermal performance curves (TPCs). In most cases, thermal extremes drive the evolution of TPCs more strongly when they cause mortality (gray lines) than when they cause sub-lethal performance reductions (i.e., injury; black lines). If cumulative damage intensifies with each incidence of an extreme, evolution selects for less thermal tolerance when extremes cause mortality and greater thermal tolerance when extremes only impact performance. The thick, light grey line depicts the case when the impacts of extremes are restricted to short term performance (i.e., no mortality or lasting performance reductions). The temperature distribution (shown as shaded gray silhouette) is derived from daily maximum temperatures in Melbourne, Australia.
127x127mm (300 x 300 DPI)

Supplementary material

Supplementary methods.

We describe the quantitative genetic model that we use to model the evolution of thermal performance curves (TPCs, modified from Buckley and Huey this issue). Our analysis extends previous work on the evolution of TPCs (Lynch and Gabriel 1987; Huey and Kingsolver 1993; Gilchrist 1995; Angilletta 2009; Asbury and Angilletta 2010). First, we estimate the time series of environmental conditions experienced by individuals across their lifespans within a population. We then use the shape of each individual's TPC to integrate performance over time. The individual performances determine fitness and selection on TPC shape.

We use a beta curve to model performance, Z, as a non-linear function of body temperature, T_b (°C):

$$Z(T_b) = \frac{[(T_b - \alpha)/b]^{\gamma/\beta - 1}[1 - (T_b - \alpha)/b]^{(1-\gamma)/\beta - 1}\Gamma(1/\beta)}{\Gamma(\gamma/\beta)\Gamma[(1-\gamma)\beta]}$$

where α, β, and γ determine the minima, breadth, and skewness of the performance curve, respectively (Asbury and Angilletta 2010). The parameter b determines the maximal breadth. We constrain the parameters to those that generate realistic curves ($-10 < \alpha < 4$, $0.05 < \beta < 0.15$, $\gamma = 0.7$, and $b = 43$), given the large diversity of curves observed among ectothermic animals (see Sunday *et al.* 2014). The area under the curve is fixed [thus excluding 'hotter is better' (Asbury and Angilletta 2010)].

We estimate fitness as the product of fecundity and survival. Fecundity is quantified as the sum of performance across time steps within a generation, and we assume low but non-zero performance outside the critical thermal limits. For those models that include mortality, thermal

stress is the sole source of mortality. We assumed that the probability of survival through a thermal stress event declined exponentially to zero between CT_{max} and 60°C. We confirmed that results were similar if survival declined linearly. The probability of an individual surviving to the end of its potential lifespan is assumed to be the product of survival across sequential time periods. We define a generation as 300 time steps. We ran the model for 200 generations (sufficient to reach equilibrium).

We use a simple quantitative genetic model to predict selection and the evolution of TPCs. We consider how two phenotypes (parameters α: minima and β: breadth) of the performance curve evolve. We use a genetic variance covariance matrix (G matrix) to account for the genetic correlation of the two parameters (vector z), and we model phenotypic evolution as $\Delta z = Gs$, where s is a vector describing selection on each of the traits (Lande and Arnold 1983). We assume genetic variances (heritabilities) of 0.7 and covariances of -0.1. The negative covariance accounts for the observation that organisms with higher thermal tolerances tend to have smaller breadths, but our results are robust to the sign of the covariance. Our estimates of the variances and covariances in the G matrix for TPCs are high and similar, respectively, relative to the limited empirical data available (Kingsolver, Ragland and Shlichta 2004), but we selected these values to speed evolution in our analysis. We used a sensitivity analysis to confirm that our results are robust to our parameterization of the G matrix.

We initialized our model with TPC minima (α) and breadth (β) that optimized performance in the initial time period in the absence of thermal extremes. We simulated 200 individuals with TPC minima (α) and breadth (β) drawn from a normal distribution with the given phenotypic mean

and variance (standard deviations= 1 and 0.02 for α and β, respectively) for each generation. We use relative fitness estimates for each individual to estimate the (unstandardized) directional selection gradients and to predict the evolutionary response to selection (change in mean phenotype) in the next generation (Lande and Arnold 1983).

Whether thermal stress occurs depends not only on microclimate variation, but also on whether individuals are able to select their preferred microclimate from the available distribution (Kearney, Shine and Porter 2009; Sears, Raskin and Angilletta 2011). We incorporated microclimate variation by drawing a value for thermal heterogeneity from a normal distribution with a standard deviation of 2°C at each time step for each individual. We incorporated behavioral thermoregulation by assuming that individuals would select the microclimate (from the distribution of available microclimates, specified by the amount of heterogeneity) at each time step that was closest to their thermal optima.

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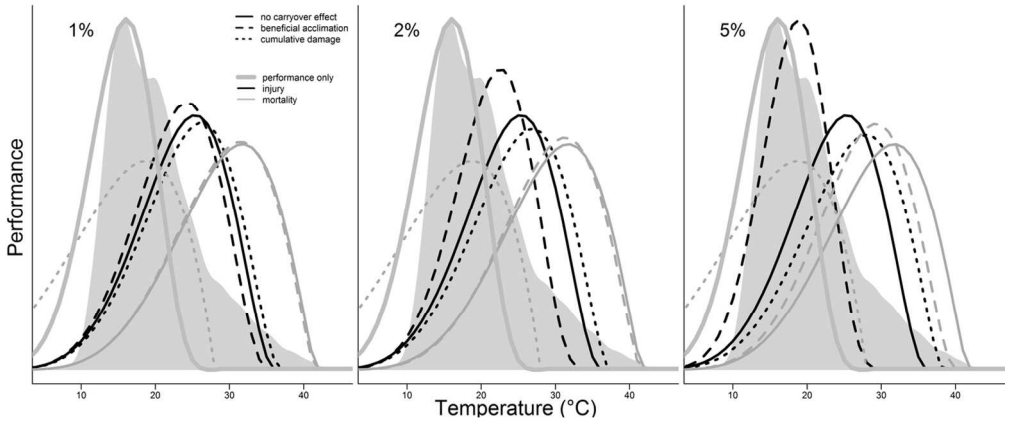
Supplementary figure

Figure S1. We examine sensitivity to the percent by which hardening or cumulative damage with each subsequent extreme impacts performance (left to right: 1% to 5%). Beneficial acclimatization (dashed lines) and cumulative damage (dotted lines) impact the evolution of thermal performance curves. We consider cases when thermal extremes cause mortality (gray lines) or permanent performance reductions (i.e., injury; black lines). The thick, light grey line depicts the case when the impacts of extremes are restricted to short term performance. The temperature distribution is derived from daily maximum temperatures in Melbourne, Australia and is shown as the shaded silhouette in grey.



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