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Brent J Sinclair

Katie E Marshall

Mary A Sewell

Danielle L Levesque

Christopher S Willett

See next page for additional authors

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Authors

Brent J Sinclair, Katie E Marshall, Mary A Sewell, Danielle L Levesque, Christopher S Willett, Stine Slotsbo, Yunwei Dong, Christopher D G Harley, David J Marshall, Brian S Helmuth, and Raymond B Huey

REVIEWS AND SYNTHESES

Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures?

Brent J. Sinclair[1]*, Katie E. Marshall[2‡], Mary A. Sewell[3], Danielle L. Levesque[4†], Christopher S. Willett[5], Stine Slotsbo[6], Yunwei Dong[7], Christopher D.G. Harley[2], David J. Marshall[8], Brian S. Helmuth[9] and Raymond B. Huey[10]

[1]Department of Biology, University of Western Ontario, London, ON, Canada
[2]Department of Zoology, University of British Columbia, Vancouver, BC, Canada
[3]School of Biological Sciences, University of Auckland, Auckland, New Zealand
[4]Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia
[5]Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[6]Department of Bioscience, Aarhus University, Aarhus, Denmark
[7]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, China
[8]Faculty of Science, Universiti Brunei Darussalam. Gadong, Brunei Darussalam
[9]Department of Marine and Environmental Sciences and School of Public Policy and Urban Affairs, Northeastern University Marine Science Center, Nahant, MA, USA
[10]Department of Biology, University of Washington, Seattle, WA, USA
‡Present Address: Department of Biology, University of Oklahoma, Norman, OK, USA
†Present Address: School of Biology and Ecology, University of Maine, Orono, ME, USA
*Corresponding Author: email: bsincla7@uwo.ca; tel. 519-661-2111 x83138; fax 519-661-3935

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Abstract

Thermal performance curves (TPCs), which quantify how an ectotherm's body temperature (T_b) affects its performance or fitness, are often used in an attempt to predict organismal responses to climate change. Here we examine the key – but often biologically unreasonable – assumptions underlying this approach; for example, that physiology and thermal regimes are invariant over ontogeny, space, and time, and also that TPCs are independent of previously experienced $T_{b.}$ We show how a critical consideration of these assumptions can lead to biologically useful hypotheses and experimental designs. For example, rather than assuming that TPCs are fixed during ontogeny, one can measure TPCs for each major life stage and incorporate these into stage-specific ecological models to reveal the life stage most likely to be vulnerable to climate change. Our overall goal is to explicitly examine the assumptions underlying the integration of TPCs with T_b, to develop a framework within which empiricists can place their work within these limitations, and to facilitate the application of thermal physiology to understanding the biological implications of climate change.

Introduction

Anthropogenic climate change is causing demonstrable and accelerating biological impacts on organisms and ecosystems, and biologists are attempting to understand and predict these impacts (Pacifici *et al.* 2015). Inevitably, these effects are mediated in large part by the behavioral and physiological responses of organisms to changing abiotic variables. Most organisms are ectotherms and thus have body temperatures (T_b – see Box 1 for a glossary of terms) that reflect their environments to varying degrees (Angilletta 2009). Extremely high or low temperatures are lethal, and temperature determines the rate of biochemical and physiological reactions. Indeed, all cellular and physiological functions, including metabolism, development, growth, movement, and reproduction, are temperature-dependent; and this has profound consequences at organismal, community, and ecosystem levels (e.g. Grigaltchik *et al.* 2012). Thus, addressing the impacts of climate change through the lens of ectotherm thermal biology allows us to draw conclusions relevant to almost all of the Earth's species.

A standard way to evaluate the ecological consequences of temperature involves (1) measuring (or predicting) actual body temperatures of ectotherms in nature and (2) determining how body temperature affects organismal-level performance (generally, the rate at which an organism can perform an ecologically-relevant activity) or fitness (Huey & Slatkin 1976). Then one can either predict instantaneous performances associated with those T_b, or, by integrating over a temperature distribution for a time interval or habitat, estimate the average performance level over a given time or habitat (see Angilletta 2009, and the references therein). More recently, this approach has also been used to predict the ecological consequences of climate warming on performance or fitness (e.g. Deutsch *et al.* 2008; Vasseur *et al.* 2014; Levy *et al.* 2015). This examination of T_b through the lens of physiological (or physiologically-mediated) responses sometimes yields counter-intuitive surprises: for example, several studies have predicted that climate warming will have relatively large and negative effects on tropical ectotherms, even though the rate of warming is slower in the tropics than at higher latitudes (Deutsch *et al.* 2008; Dillon *et al.* 2010; Thomas *et al.* 2012; Sunday *et al.* 2014).

Measuring instantaneous physiological rates (‘performance’) across temperature generally yields a curve where performance (assumed to be a proxy for fitness) rises slowly with temperature up to a maximum level (T_{opt}), and then drops rapidly (Fig. 1). These Thermal Performance Curves (TPCs) describe how T_{b} affects an ectotherm’s performance or fitness (Huey & Stevenson 1979) over the range of T_{b} for which performance is positive (i.e. between the critical thermal minimum and maximum, CT_{min} and CT_{max}). In studies with ecological applications, TPCs typically quantify whole-organism performance (e.g., speed, stamina, feeding rate, or growth) or sometimes fitness proxies (e.g. reproductive output), because such integrative, higher-level, traits are more directly related to ecological performance than are lower-level ones such as enzyme activity.

Mapping T_{b} onto performance provides an intuitive heuristic model of impacts of temperature or temperature change on organism physiology and ecology. This mapping is not, however, without hazards. Here we explore assumptions and complications associated both with quantifying T_{b} and TPCs, and specifically when integrating them to predict impacts of climate change. These factors can fundamentally alter predictions of the likely impacts of climate change, but our initial goal is to identify the assumptions underlying TPC-based models, and to encourage analyses of how sensitive the models are to those assumptions. Our central conclusion is that the TPC-T_b approach – despite many limitations – remains a useful exploratory tool for evaluating responses to climate change.

Using TPCs to predict the consequences of climate change

In principle, TPCs and T_b distributions can be used to predict the performance or fitness consequences of an organism's thermal environment. First, one empirically estimates how fitness, w, changes instantaneously with T_b, giving $w(T_b)$. Next, one estimates the frequency distribution of body temperatures, $p(T_b)$, experienced by the animal during some time period. The total fitness (W) in a given environment can then be integrated via Eqn. 1, which is, in effect, a rate summation of fitness over T_b (Huey & Slatkin 1976; Deutsch *et al.* 2008; Vasseur *et al.* 2014):

$$W \sim \int_{CT_{\mathrm{min}}}^{CT_{\mathrm{max}}} [w(T_{\mathrm{b}}) \,.\, p(T_{\mathrm{b}})]\, dT_{\mathrm{b}} \qquad (1)$$

Fitness will approach a maximum if most T_b match the optimal T_b (T_{opt}, see Fig. 2), which should (theoretically) be the preferred temperature (but see Martin & Huey 2008). A shift in the mean of $p(T_b)$ – caused by behavior, seasonality, habitat selection, or climate change – can increase, have no impact, or decrease total fitness, depending on the magnitude, direction, and position of the shift relative to T_{opt} (Huey 1991). Similarly, a shift in the variance or skewness of $p(T_b)$ will also have positive or negative effects on W, again depending on the magnitude and position of the shift relative to T_{opt} and to the degree of thermal specialization versus generalization (Angilletta 2009; Vasseur *et al.* 2014), in part as a consequence of Jensen's inequality (discussed below).

Equation 1 evaluates fitness as a function of the T_b experienced by an ectotherm during some time period, but T_b can sometimes reach or exceed the CT_{max} – for example, if temperatures warm, or if the animal moves into the sun – with deleterious and potentially lethal consequences. Because the TPC is asymmetric, T_{opt} is much closer to the CT_{max} than it is to the CT_{min} (Fig. 1). A risk-avoidance hypothesis (Martin & Huey 2008) proposes that ectotherms should avoid T_b that approach CT_{max}: thus they should maintain an ample "thermal safety margin (TSM)." [Note: TSM has been defined in several ways. In Fig. 1 and Box 1, we use the distance between the optimal T_b and the CT_{max}]. The smaller the thermal safety margin in a given environment, the greater the likelihood that an organism will overheat (and possibly die) as climate warms. Because among-species variation in CT_{max} is relatively small, tropical species – assumed to experience relatively stable, warm, temperatures (Janzen 1967) – should have very small TSMs and therefore be disproportionately affected by small increases in mean temperature with climate change (Deutsch *et al.* 2008). Parallel arguments have extended this concept to other stenotherms, for example polar fishes (Peck *et al.* 2010). Importantly, CT_{min} and CT_{max} bound the TPC, but are not necessarily survival limits, especially during short-term exposures. For example, freeze-tolerant sub-Antarctic *Pringleophaga marioni* caterpillars stop moving at around -0.6 °C, but only die at temperatures below c. -7.5 °C (Klok & Chown 1997). Some intertidal gastropods lose mobility at CT_{max}, but still survive brief exposures to higher temperatures (e.g. Marshall *et al.* 2015).

The curvilinear relationship between performance and temperature over much of the TPC (Fig. 1) means that the effects of small changes in temperature can be small, negligible, or large, depending on where on the TPC those changes occur (Jensen's inequality – see Ruel & Ayres 1999). Jensen's inequality has two significant implications for ectotherms under climate change. First, thermal variability becomes a central determinant of ectotherms' responses to environmental change independent of changes in mean temperature (e.g. Helmuth *et al.* 2014; Vasseur *et al.* 2014; Colinet *et al.* 2015). Second, because metabolic rates increase exponentially with temperature below the inflection point, for a given shift in temperature the metabolic rates of ectotherms in regions with high mean temperatures (i.e. the tropics) may increase more than those of ectotherms in regions with a relatively low mean temperature (e.g. terrestrial Arctic habitats), even though temperature increases in the tropics have been relatively small (Dillon *et al.* 2010). By contrast, Kingsolver *et al.* (2013) and Vasseur *et al.* (2014) argued that temperate species may be more vulnerable to climate warming than are tropical species because of higher thermal variability in temperate zones, which increases the incidence of lethal temperatures, despite lower mean temperatures. Variability can be important even at non-lethal temperatures in temperate species; for example, driving evolution of metabolic suppression in butterflies (Williams *et al.* 2012).

Do TPCs really estimate fitness?

The relationships between T_b and fitness in Eqn. 1 are simple and appealing. If one knows the TPC and how climate change will affect $p(T_b)$, one can predict the fitness consequences of climate change for an ectotherm. However, fitness is notoriously hard to define, let alone to measure. Classical life history measures of fitness (e.g., net reproductive rate, R_o; intrinsic rate of population growth, r) must be measured at least over an organism's lifespan (Huey & Berrigan 2001; Thomas *et al.* 2012). Not surprisingly, actual measurements of the thermal dependence of fitness have generally been limited to short-lived taxa in the laboratory or to organisms studied by – possibly hypothetical – biologists with long careers and reliable funding. Such data exist as life tables (age-specific table of survival and reproduction) primarily of economically-important insects raised at multiple temperatures (Huey & Berrigan 2001). Importantly, TPCs for *r* and R_o have different shapes, even when based on the same life table, because *r* is inversely related to generation time, which in turn decreases at high temperatures: consequently, T_{opt} for *r* is often higher than that for R_o (Huey & Berrigan 2001), and analyses using *r* vs. R_o can yield conflicting predictions (Deutsch *et al.* 2008; Kingsolver *et al.* 2011). These life table studies also require exposing animals throughout their lives to fixed temperatures (see Assumption 9 in Table 1). Two problems arise here. First, fixed temperature exposures are inappropriate if life stages live in different micro-environments and thus experience different body temperatures in nature, which is true for insects and many other taxa (Kingsolver *et al.* 2011; Colinet *et al.* 2015; Levy *et al.* 2015). Second, long exposure to fixed temperatures may induce pathologies, especially at high temperature (Kingsolver & Woods 2016).

Because of the above issues, an instantaneous measure of performance, such as locomotor speed or feeding rate, is often used as a proxy for $w(T_b)$ (Assumption 1, Table 1; Figures 3, 4, 5). Often the choice of performance traits for TPC analyses is driven by expediency, rather than by validated links to fitness. Importantly, TPCs estimated for different traits can differ markedly even in a single species (Fig. 3), which means that contrasting conclusions about fitness could easily be derived from TPCs for different traits acquired on the same organism. Maximal sprint speed has been measured across the most taxa, but its relationship to fitness is rarely established (Miles 2004). Feeding rate can determine an organism's ability to meet and exceed metabolic demands but, above some threshold, mechanical limits to food processing or physiological limits to absorption mean that additional food does not necessarily increase fitness (Riisgard 2001), and it is unclear where this threshold occurs relative to T_{opt}. Trait differences may arise from physical constraints or evolutionary and behavioral selection of different thermal regimes that mean they have been optimized for specific $p(T_b)$; for example locusts enhance digestion by selecting high temperatures after a meal, but choose cooler regimes to reduce energetic costs when starved (Clissold *et al.* 2013). TPCs can also shift with food resources (Fig. 6a; Brett 1971), and are not, as Eqn. 1 implies, fixed. One approach to resolve this issue may be to integrate a composite panel of TPCs that use different $w(T_{\mathrm{b}})$ relationships for different activities, habitats, or times of day.

Finally, some temperature-dependent traits may be poor proxies for fitness. For example, although resting or standard metabolic rates increase with temperature, higher rates indicate higher energetic expenditures as well as higher activity, and may not therefore translate to higher fitness, particularly during non-feeding life stages (Clarke 1991). Thus, identifying the most relevant fitness proxies is necessary when parsing contrasting – or even contradictory – signals from different traits (e.g. Fig. 3).

Breaking down *w(Tb)*: What are the implicit and explicit assumptions of using TPCs to predict fitness?

Equation 1 provides a simple way to conceptualize how organismal thermal sensitivity (TPC) and body temperature map to organismal fitness (W). However, doing so makes a number of assumptions about the relationship between temperature and fitness. In particular, biologists tend to assume that their chosen trait reflects fitness (Assumption 1 in Table 1), that TPCs are evolutionarily fixed (Assumptions 2-5 in Table 1), that the well-documented physiological adjustments to temperature can be ignored (Assumptions 6-9 in Table 1), and that temperature is the primary driver of fitness (Assumptions 10 & 11 in Table 1). Below we critically address each assumption.

Are TPCs invariant in space and time?

Macrophysiological analyses generally assume that the TPC of a species (or sometimes of an Order or Class) can be adequately described by a single curve. This is valid only if numerous – and unlikely – conditions are met (Assumptions 2-11 in Table 1). The shape, maximum, limits, and breadth of TPCs can vary with habitat, nutritional state, developmental stage, and acclimation history (Figures 4, 5, 6). In addition, individuals within a population may differ significantly, due to both genetic and non-genetic causes (Kingsolver *et al.* 2011; Logan *et al.* 2014; Assumption 10 in Table 1).

The use of 'one species, one TPC' also assumes that the TPC is invariant over both geographical range and evolutionary time (Assumptions 3 and 4 in Table 1). In fact, the thermal sensitivity of ectotherms sometimes varies markedly across their range, often in concert with local conditions. This variation can alter predictions of population dynamics at range edges under climate change (Pearson *et al.* 2009). For example, thermal tolerance of barnacles is higher in subpopulations that experience more extreme temperatures (Schmidt *et al.* 2000), and thermal tolerances can vary widely among insect populations (reviewed by Sinclair *et al.* 2012). This local adaptation illustrates the evolutionary potential of thermal biology to shift over relatively short time scales –

less than a century in the case of the cabbage white butterfly, *Pieris rapae*, in North America (Kingsolver *et al.* 2007). Thus, natural selection might conceivably alter $w(T_b)$ for species with short life cycles by the 2050 and 2100 dates used for most climate change projections.

The capacity for $w(T_b)$ to evolve in this timeframe will vary among taxa, habitats, and traits (Hoffmann & Sgrò 2011), and will also depend on the extent to which climate change affects $p(T_b)$ – see below. The importance of evolution in altering responses to climate change is widely acknowledged (Munday *et al.* 2013), but unfortunately the sensitivity of predicted outcomes to either evolution or plasticity has rarely been incorporated into models (but see Dowd *et al.* 2015 for an example). Sensitivity analyses will be required to develop 'rules' about how robust predictions are to evolutionary change and (conversely) how much evolutionary capacity or plasticity is necessary to offset climate change impacts.

Do TPCs reflect the realities of the thermal environment?

TPCs for fitness traits are typically constructed using exposure to fixed temperatures, but extrapolating TPCs to field conditions can be complicated by thermal environments that are often highly heterogeneous in space and time, affecting $p(T_b)$ (see below). Thus, both $w(T_b)$ and the experiments we use to derive TPCs carry important assumptions that must be accounted for when using TPCs to derive predictions about the thermal performance of ectotherms in nature.

Importantly, temperature exposures in nature vary in duration, and the duration of exposure to a given temperature can determine performance and fitness. For example, a 30-min exposure to 36 °C initiates a protective molecular cascade (the 'heat shock response') in *Drosophila* *melanogaster*, but exposure to 29 °C for more than a few hours renders flies sterile (David *et al.* 2005). These duration effects are particularly significant at high temperatures, where performance usually declines with exposure time (Rezende *et al.* 2014). Even so, mortality and damage accumulation can also result from long exposure to low temperatures (Nedvěd *et al.* 1998; Rezende *et al.* 2014). Such duration effects imply that *TPC*s are temporally dynamic, but this has been generally ignored in models (Assumptions 6, 7, 11 in Table 1; but see Kingsolver & Woods 2016).

Animals in nature usually experience temperatures in a certain order; a change in T_b from temperature x to temperature $x+3$ necessarily involves exposure to temperatures $x+1$ and $x+2$. Thus, thermal regimes usually do not shift suddenly, except in instances where the animal moves from shade to sun or air to water, or in subtidal environments subject to strong tidal currents, which all can lead to abrupt changes in T_b (Leichter *et al.* 2006). In experiments where performance of individual animals is measured at multiple temperatures, the sequence of body temperatures is usually randomized (although the highest temperature often comes last to avoid any heat shock response affecting performance at other temperatures; e.g. Williams *et al.* 2012). These randomized protocols have some empirical support: for example, thermal sensitivity of instantaneous cricket metabolic rate calculated from ramped, ordered or randomized temperature did not significantly differ (Lake *et al.* 2013). Nevertheless, animals in nature have had prior thermal experience that is largely unaccounted for in TPCs (Assumption 6 in Table 1).

Prior thermal experience can modify the TPC directly. For example, acclimation responses can substantially modify the shape and position of the entire TPC (e.g. Fig. 5), including thermal limits (Angilletta 2009). Although the broad physiological and biochemical mechanisms underlying these changes are reasonably well-understood, predicting how TPCs will shift is challenging, even in broad geographic comparisons (Somero 2010). Tropical *Drosophila* appear to have sufficient plasticity to maintain an adequate thermal safety margin (Overgaard *et al.* 2011), whereas porcelain crabs do not (Stillman 2003). Predicting TPCs is made even more complicated by cross-generation effects on TPCs. For example, female blow flies exposed to relatively warm autumn temperatures produce larvae with reduced cold hardiness, which likely reduces overwinter survival (Coleman *et al.* 2014). The capacity for plastic responses to changing temperatures can also depend on the rate of temperature change: emerald ash borer prepupae have relatively high heat tolerance when shifted slowly to a high temperature, because slow warming allows them to mount a heat shock response (Sobek *et al.* 2011). Whether or not acclimatization is an effective strategy in nature will thus depend on how temporally autocorrelated thermal regimes are over the scale of days, i.e. whether preparing for an extended heat wave or cold snap is an effective use of physiological resources.

Both the order of thermal exposure and the rate of temperature change can affect $w(T_b)$, but neither is usually accounted for in models (Assumptions 6 and 8 in Table 1), even though both vary in nature. Plastic responses to temperature fluctuations will likely bear costs and elicit trade-offs, not just as simple shifts in the instantaneous value of $w(T_b)$, but in terms of long-lasting accumulation of fitness. This will particularly be the case when organisms are exposed to temperature extremes. For example, the heat shock response requires energy for the synthesis and ATP-dependent activity of heat shock proteins (Feder & Hofmann 1999), and recovery from being cooled to below the CT_{min} has a measurable metabolic cost in insects (MacMillan *et al.* 2012).

Finally, thermal regimes in nature often repeat themselves (but see above for exceptions); for most habitats, diel thermal cycles mean that an ectotherm that lives for more than a few hours will be exposed to repeated warm-cold fluctuations (Colinet *et al.* 2015). Given that prior experience can modify the TPC, the degree to which TPCs remain constant across multiple thermal cycles will depend in part on the temporal autocorrelation of the environment, which may be modified with climate change (Assumption 6 in Table 1). Fitness can decline because of repeated exposure to deleterious temperatures – in insects and lizards, this effect may be more important than the duration or intensity of exposure to extreme temperatures (Kearney *et al.* 2012; Marshall & Sinclair 2015). Conversely, thermal cycles under permissive temperatures often increase growth rates (and presumably fitness; Colinet *et al.* 2015). Overall, a predictive understanding of how thermal fluctuations affect ectotherm fitness is still elusive (Kingsolver *et al.* 2013; Vasseur *et al.* 2014; Colinet *et al.* 2015), and empirical responses might well prove idiosyncratic.

Thus, in reality, $w(T_b)$ is not a fixed curve but a shifting multi-dimensional envelope with an explicit temporal history. Estimates may need to incorporate threshold-crossing events plus duration and frequency of exposure to stressful temperatures (Assumptions 7 and 9 in Table 1). The consequences of exposure to temperature extremes have been included in models in several ways. Deutsch et al. (2008) assumed that fitness was temporarily zero when $T_{\mathrm{b}}>CT_{\mathrm{max}}$ (or $<CT_{\mathrm{min}}$), Kingsolver et al. (2011) assumed that individuals died under these same conditions, and

Buckley and Huey (2016) assumed that survival declined exponentially to zero between the CT_{max} and 60°C. Roitberg and Mangel (in press) have proposed splitting the TPC in two, with fitness costs accumulating (and the $w(T_{\mathrm{b}})$ curve modified) after exceeding CT_{max}, but not the CT_{min}. This latter approach reflects modifications to the TPC by the heat shock response (Feder & Hofmann 1999), and perhaps provides a template for how other thermal-history-based modifications to the TPC can be modelled. Alternatively, perhaps we need to shift entirely from a TPC approach to a time series model that reflects the time × sequence × duration × temperature interaction implicit in thermal biology in nature – Woodin et al. (2013) begin to take this approach by applying a time component when $T_{\mathrm{b}}>CT_{\mathrm{max}}$. In the short term, determining the relative importance of these components could allow the key drivers of the $w(T_{\mathrm{b}})$ relationship to be identified and incorporated. For example, the number of cold exposures has a bigger effect on fitness than either the duration or intensity of those exposures in overwintering spruce budworm: thus, a term quantifying exposure-number could account for most effects of thermal variability on this species (Marshall & Sinclair 2015).

Beyond temperature: TPCs in a multi-stressor world

Environmental physiology of ectotherms often focuses on temperature as a "master variable" that dominates the performance, survival, and fitness of organisms (Assumption 11, Table 1). Nevertheless, interactions involving numerous other environmental and biological factors can alter the shape of an organism's TPC and thus how an organism relates to its thermal environment (e.g. Fig. 4; Denny *et al.* 2009; Todgham & Stillman 2013; Gunderson *et al.* 2016). Furthermore, performance curves can just as readily be constructed with respect to other environmental variables such as salinity, pH, and water vapor deficit, and to other anthropogenic stressors, such as pollutants, each of which can modify the effect of temperature on performance (Gunderson *et al.* 2016). Some of these abiotic factors are themselves temperature-dependent; for example, oxygen saturation and $p\mathrm{CO}_2$ for aquatic organisms (Deutsch *et al.* 2015; Gunderson *et al.* 2016). When such interactions occur, the combined effect of two variables usually cannot be predicted merely by summing the individual effects from single parameter experiments. Non-additive (synergistic) or even antagonistic outcomes in multiple stressor scenarios appear to be the norm, and varying a larger number of environmental parameters yields more substantial effects (Denny *et al.* 2009; Todgham & Stillman 2013; Brennan & Collins 2015; Deutsch *et al.* 2015). Fractional factorial designs may be required to deal with multiple factors (Porter *et al.* 1984).

In most cases, the physiological mechanisms underlying non-additive outcomes in multi-stressor scenarios are not yet fully understood. However, one proposed mechanism linking two stressors in a predictive fashion is oxygen and capacity limited thermal tolerance (OCLTT), which relates performance both to temperature and to the supply of oxygen to the tissues, and therefore to aerobic scope (Pörtner 2010). The generality of OCLTT is debated (e.g. Verberk *et al.* 2016). For example, in contrast to the OCLLT, where extreme temperatures reduce the capacity to deal with a second stressor (reduced oxygen), exposure to thermal extremes can also increase tolerance to other stressors, including hypoxia and hyperoxia, leading to cross-tolerance among multiple stressors (Todgham & Stillman 2013). Nevertheless, the OCLTT approach is an excellent example of a mechanism-based integration of two interacting stressors, and has been used to generate global-level predictions about responses of some aquatic species to climate change (Deutsch *et al.* 2015)..

Biotic interactions and TPCs

Because of high interspecific variability in thermal performance, climate change is expected to result in "winners" and "losers" (Somero 2010). When performance differs among ecologically-important species such as structuring species, ecosystem engineers, and keystone predators, differential vulnerability among interacting species can translate into differential vulnerability of entire assemblages (Monaco & Helmuth 2011; Dell *et al.* 2014). Similarly, TPCs can be modified by interactions among species: shifts in food abundance (e.g. via predator-prey interactions, or competition) can modify the TPC (Figure 6); non-consumptive effects ('fear of being eaten') can reduce foraging success and efficiency, or elicit other physiological costs (Rovero *et al.* 1999; Nelson *et al.* 2004); and parasites and pathogens can induce direct physiological costs (Vernberg & Vernberg 1963) that might modify the TPC. Community interactions can themselves be determined by temperature, creating feedback loops between TPCs and interspecific interactions. For example, elevated temperatures can increase or decrease foraging rates of predators, depending on whether temperature increases occur below or above an organism's T_{opt} (Monaco & Helmuth 2011).

Animals carry with them communities of microbes that can affect behavioral and physiological phenotypes (McFall-Ngai 2015). Although the effect of symbionts on TPCs has not, to our knowledge, been directly explored, there is substantial evidence that symbionts can modify thermal limits. For example, mutations in endosymbiotic *Buchnera* determine the thermal tolerance of their aphid hosts (Dunbar *et al.* 2007), and thermally-tolerant strains of endosymbiotic *Symbiodinium* increase the thermal tolerance of their host corals by more than

1°C (Berkelmans & van Oppen 2006). Interactions between immunity and pathogens will also help to shape the TPC; for example, crickets deactivate low temperature immunity during cold acclimation in a trade-off with other physiological activities that contribute to $w(T_b)$ (Ferguson *et al.* 2016). Thus, $w(T_b)$ and $p(T_b)$ can be altered by numerous interactions involving hosts and symbiotic or pathogenic microbiota.

Breaking down $p(T_b)$

Global models of ectotherm responses to climate change depend on the relationship between fitness and T_b, and thus the distribution of T_b animals experience, $p(T_b)$. Although T_b has been extensively measured and modelled for animals, empirical T_b distributions are seldom incorporated in global-scale analyses, which tend to substitute large-scale meteorological air- or water-temperature datasets for $T_{b,}$ and thus ignore behavioural thermoregulation and micro-scale environmental variation (Kearney *et al.* 2009; Sears *et al.* 2011; Potter *et al.* 2013). In the simplest cases, such as a soil ectotherm that does not thermoregulate, $p(T_b)$ will be very close – if not identical – to the distribution of operative temperatures, $p(T_e)$, measured in the soil. However, the assumption that $T_e=T_b$ is often extended into heterogeneous situations, to animals with significant capacity to regulate T_b, or to animals whose T_b is affected by morphology, thermal inertia, or surface coloration; in these situations, instantaneous $T_e \neq T_b$. Moreover, behavioral thermoregulation and physiology can decouple T_b from T_a in space and time (Fig. 7; Sunday *et al.* 2014). Interspecific interactions can also shift $p(T_b)$: grasshoppers exposed to avian predators move to lower (cooler) positions in the vegetation (Pitt 1999). Thus, properly quantifying p(T_b) is essential for improving the accuracy and precision of conclusions about ectotherm responses to climate change.

Many ectotherms can actively behaviorally thermoregulate to maintain a $p(T_\mathrm{b})$ with a mean and variance that are substantially different from $p(T_\mathrm{e})$. Behavioral thermoregulation can be highly active, such as in lizards that shuttle from shade to sun (Kearney *et al.* 2009), or more passive, such as the periwinkle *Echinolittorina peruviana*, which orients its narrower sides towards the sun on hot days (Muñoz *et al.* 2005). There are hard limits to plasticity of CT_max in at least some species (Stillman 2003), which means that behavioral thermoregulation will be essential for survival of tropical stenotherms with limited plasticity and small TSMs (Kearney *et al.* 2009; Sunday *et al.* 2014).

In many animals, T_b can be measured directly in nature and thus generate accurate values for $p(T_\mathrm{b})$. Methods for estimating T_b of free-ranging animals in nature include telemetry (e.g. Mitchell *et al.* 1997; Briscoe *et al.* 2014) as well as instantaneous measurements of T_b in freshly-captured animals (e.g. 'grab and stab' in insects; Bartholomew & Heinrich 1973). Telemetry does not interfere with an organism's thermoregulation and movements, and allows measurements during both active and inactive periods, but can only be used on species large enough to surgically implant a sensor. However, small data loggers can also be attached or implanted, but must later be collected (Davidson *et al.* 2003).

Alternatively, $p(T_\mathrm{b})$ can be estimated via physical models ('biomimetic sensors'), such as 'robomussels'(Helmuth *et al.* 2002): such models can accurately mimic the physical properties – and thus equilibrium heat exchange – of specific organisms in a given microclimate (Bakken 1992). Most such models are dry-skinned, so assume negligible evaporative heat loss (but see

Köhler *et al.* 2011; Monaco *et al.* 2015), but do account for size, shape, and color in generating maps of T_b. Automatic recordings from biomimetic sensors can easily provide long-term (even multi-year) records (Helmuth *et al.* 2010); but (except for completely sessile organisms such as intertidal bivalves), they necessarily ignore behavioral thermoregulation. Other approaches deploy biomimetics in multiple potential habitats, and then estimate realized $p(T_\mathrm{b})$ using a series of behavioral rules, such as optimization of performance or avoidance of extremes (e.g. Monaco *et al.* 2015).

An alternative (or adjunct) to using direct biomimics to estimate $p(T_\mathrm{b})$ is to develop biophysical (e.g. heat budget) models that predict T_b from environmental variables (e.g. wind speed, air temperature, and solar radiation) and the physical properties of the organism, and then use climate projections to develop an overall heat budget and thus estimate $p(T_\mathrm{b})$ (Kearney *et al.* 2009). These relationships are not necessarily simple: the size, color, morphology, and orientation of organisms alters heat exchange with their environments (and thus T_b); the thermal properties of materials vary (e.g. shell has a lower specific heat capacity than wet tissues), as do the properties of surfaces presented to the environment. Similarly, body size can buffer rapid changes in temperature (larger animals have higher thermal inertia), but even large animals can modify heat exchange via thermal windows such as large bills, fins, or ears (e.g. Tattersall *et al.* 2009). To account for all of this variety, biophysical models must be developed in a species-specific (and maybe even a life-stage-, morph-, or sex-specific) manner, making it difficult to extrapolate broadly in space, time, or across species.

Can we predict future $p(T_\mathrm{b})$?

Global-scale predictions of responses to climate change require prediction of future $p(T_b)$. This is theoretically possible via biophysical models (Kearney *et al.* 2009), but changes in cloud-, plant-, and snow-cover could easily modify thermal environments, and thus $p(T_b)$, even without changes in climatic temperature. One approach to understanding how $p(T_b)$ may change is to observe how $p(T_b)$ changes in response to latitudinal or altitudinal gradients as an analogue to changes in time (space for time substitution) (Halbritter *et al.* 2013). However, such extrapolation is inherently problematic, because $p(T_b)$ may not change in time in the same way it does in space at present, and confounding factors, such as variation in cloud or vegetation cover or in radiation loads, are unaccounted for in a space-to-time substitution. In fact, empirical data show that geographic and altitudinal patterns do not always conform to simple gradients due to the over-riding importance of local environmental conditions. Thus, elevation and latitude can be misleading metrics of thermal stress in the future (Helmuth *et al.* 2002; Pearson *et al.* 2009), and they should be used as proxies only with appropriate caution.

A simplistic (but common) approach is to use predicted changes in average air temperature (e.g. "+2 °C" for a given site) to predict future T_b and thus physiological responses and organismal vulnerabilities (Helmuth *et al.* 2014). However, such an approach ignores regional and temporal variation, the importance of extremes (weather events), or changes in variability regimes embedded within large-scale climate (Denny *et al.* 2009). In many cases, ecosystems are already experiencing local and short-term increases in temperature that exceed the projected changes in global averages over the next century. For example, sea surface temperatures in the Gulf of Maine are increasing faster than in the global ocean (Mills *et al.* 2013), and terrestrial temperatures are increasing significantly faster in the Arctic and Antarctic than in other biomes

(Nielsen & Wall 2013). Thus, any TPC-based predictions of the responses of ectotherms to climate change are only as good as the assumptions underlying the 'future climate' data input into the model – an issue that has, in itself, received extensive discussion (see, e.g., Helmuth *et al.* 2014; Pacifici *et al.* 2015).

Thus, although $p(T_b)$ has been explored, the temporal and spatial scale best used in ecological models remains subject to debate (Sears *et al.* 2011; Potter *et al.* 2013). Predicting $p(T_b)$ at a global scale will likely require a combination of actual measurements, biomimetic data and biophysical models that incorporate seasonal and ontogenetic variation with behavioral and microclimate modification (e.g. Levy *et al.* 2015). Crucially, these global-scale corrections of $p(T_b)$ will be needed to generate predictions by region or species. One way to generalise such predictions may be to develop models for particular combinations of animal and microclimate characteristics, and then conduct additional analyses to apply these models to appropriate location/species combinations.

Putting Humpty Dumpty back together again

In any science, a general theoretical approach to a problem can be destroyed by piling up multiple objections to its implicit and explicit assumptions, or by enumerating counter examples. With respect to TPCs and the modelling approach exemplified in eqn. 1, we could allow thousands of cuts – some are discussed above – to kill this idea. However, we currently do not see an obvious substitute for the TPC approach. Consequently, we suggest that the best way forward is to modify eqn. 1 to make it more robust, functional, and sensitive to real world issues.

Thus, our goal now is to put the Humpty-Dumpty of TPCs (which we and others have now gently smashed) back together again.

First, Table 1 demonstrates many challenges with measuring and interpreting $w(T_b)$, particularly in integrating across multiple levels of biological organization. These need to be resolved through laboratory investigations (e.g. using *Drosophila* or other models) to better understand the sources and consequences of inter-individual variation in TPCs, coupled with field-based studies to better understand *TPC*s in nature. A key goal will be to determine how best to incorporate and predict plastic and evolutionary capacities as well as within- and among-population variation in *TPC*s. Also, we need to better understand the relationship between instantaneous performance (the subject of most TPCs) and long-term fitness, for example via longitudinal studies in nature, or via molecular or physiological markers of performance characteristics of wild-caught animals. Such an approach will need to recognize that generalizations will not apply to all species and traits. In addition to existing "model systems" (for which we have considerable knowledge of their genetics, physiology, phylogeny and ecology), additional foci should include: ecologically important species that have a disproportionate impact in communities (such as keystone predators and habitat-forming species); invasive species and disease vectors; and species that provide important ecosystem services. Second, temperature is an effective master regulator, and is a good place to start, but we need to evaluate the impacts of multiple interacting stressors plus interactions with the microbiome, all of which modify predictions derived from TPCs. Third, we need to evaluate our estimates of contemporary $p(T_b)$, and consider how this affects our ability to predict future $p(T_b)$. For example, thermal microrefugia may prevent local extinctions (Potter *et al.* 2013), if those refugia persist under climate change (Lima *et al.* 2016). Likewise, we need a better understanding of how anthropogenic activities will affect key modifiers of microhabitat, such as shading, air and water flow or quality, and precipitation.

Many opportunities exist for modifying our existing TPC models when making global-scale predictions (Table 1). Mechanistic models of species' distributions are already emerging that account for some of the assumptions we have identified (see Maino *et al.* 2016 for a recent summary). In terms of predicting $w(T_b)$ some cases (e.g. fluctuating temperatures, multiple stressors, biotic interactions) will require more empirical data to determine the extent to which TPCs are predictable and generalizable. In many cases, however, models can and should be adjusted to better account for assumptions we already know to be invalid, such as ontogenetic variation (Kingsolver *et al.* 2011; Levy *et al.* 2015). Earth System Models in the plant sciences, which predict photosynthetic responses to climate change on a global scale, demonstrate that large, complex, trait-based approaches are possible and can be (broadly) successful (Rogers 2014). In the long term, we may realise that the current TPC model, which is based on instantaneous performance $p(T_b)$, is flawed, but we do not yet know whether its flaws are fatal and require us to move to a temperature-plus-time-series (and possibly -plus-energetics) approach to account for the complex temporal nature of thermal biology. However, for now, we believe that TPCs offer us at least an opportunity to explore climate change with broad strokes.

Ultimately, the TPC-based approach is an heuristic starting point for evaluating the biological impacts of environment and environmental change. Understanding $w(T_b)$ is clearly important, but relating fitness to temperature will be difficult. Similarly, predicting $p(T_b)$ is essential, but currently flawed. Even so, the distribution of body temperatures is not the only physiological variable that matters. Moreover, $w(T_b)$ and $p(T_b)$ aren't independent: the T_b history can modify $w(T_b)$. This is biology. The way forward is thus either to embrace such complications into our theoretical models, or to find whether the biological signal of climate change is sufficiently strong to overpower these complications. Each of the assumptions explored here can be converted into testable hypotheses and then explored in empirical sensitivity analyses, which will provide insights into how much detail is needed and what can be ignored, reducing the uncertainty in the TPC-based approach to predicting the biological impacts of climate change. Simple models like TPCs may therefore have a future, provided we acknowledge the inherent assumptions.

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Table 1. A summary of assumptions often made in evaluations of the relationship among the environment, body temperature, and fitness, and on their consequent hypotheses and predictions. Many of these have already been shown to be false as general rules; see text for discussion.

Assumption	**Hypothesis**	**Prediction(s)**
Relationship between trait and fitness		
1 The trait *x* measured reflects fitness	Performance of trait *x* is directly correlated with *W*	Individuals with a greater value of *x* have greater lifetime reproductive output.
Variation in developmental and evolutionary contexts		
2 Thermal performance does not change with development and reproduction	The form of the *TPC* is static through an individual's lifetime if the environment is static	Measured *TPC*s will not change during development/ maturation in a predictable manner for a given species
3 Thermal performance does not vary across a species' geographic range	$w(T_b)$ is invariant within a species	No local adaptation of *TPC*s
4 TPCs will not change with climate change in the short term	$w(T_b)$ does not evolve rapidly	No rapid evolution of *TPC*s
5 TPCs can be extrapolated to higher taxonomic levels	$w(T_b)$ is phylogenetically constrained	Hierarchical taxonomic structuring of *TPC* properties
Physiological variation		
6 Prior thermal experience does not matter	$w(T_b)$ is invariant with respect to prior temperature exposure	1. *TPC*s are independent of the order of temperature exposure 2. *TPCs* do not change with repeated exposures
7 Extreme exposures do not matter	$w(T_b)$ does not change if temperature cycles cross physiological thresholds	1. TPCs will not change after pre-exposure to temperatures above the T_{opt} or close to the CT_{max} and CT_{min} 2. *TPC*s will not vary even with multiple exposures to a thermal cycle
8 Rate of temperature change does not matter	$w(T_b)$ is invariant with respect to rate of temperature change	*TPC*s will not differ between rapid or slow temperature transitions
9 Duration of temperature exposure does	$w(T_b)$ estimates are robust to the	*TPC*s will not differ when calculated from long or short

	not matter	duration of thermal exposure	exposure to each temperature
Temperature as the primary driver of fitness			
10	Variation in thermal performance due to stochastic variation or biotic impacts (e.g. in parasitism, microbiota, and nutrition) can be ignored	The majority of inter-individual variation in $w(T_b)$ is heritable	1. Heritable variation in *TPC*s exceeds plasticity 2. *TPC*s and $p(T_b)$ are not affected by inter- and intra-specific interactions
11	Temperature is the only environmental parameter whose changes affect fitness	$w(T_b)$ is invariant across gradients of additional abiotic factors	The *TPC* will not change with variation in non-thermal environmental parameters.

Text Box 1: A Glossary of Thermal Biology Terms

CT_{max} – critical thermal maximum, upper thermal limit of performance.

CT_{min} – critical thermal minimum, lower thermal limit of performance.

OCLTT – oxygen- and capacity-limited thermal tolerance; hypothesis that thermal performance at high and low T_{b} is limited by oxygen availability.

T_{a} – ambient temperature, an imprecise term often used as a synonym for air temperature, but can also reflect microhabitat temperature or the (measured) temperature of an animal's immediate surroundings.

T_{b} – body temperature (usually core).

T_{br} – breadth of thermal performance.

T_{e} – operative temperature – equilibrium T_{b} of a specific organism in a specific microenvironment, assuming no metabolic heat increment or evaporative cooling.

T_{opt} – optimum body temperature, at which performance is maximal.

TPC – thermal performance curve; depicting performance as a function of T_{b}

T_{p} – preferred (selected) body temperature, often measured in a laboratory thermal gradient.

TSM – thermal safety margin; various definitions are in use, but *TSM* is generally inversely proportional to the risk of an animal experiencing temperatures above CT_{max}. Here we define it as the difference between T_{opt} (or maximum T_{b} in the field) and CT_{max}.

$p(T_{\mathrm{b}})$ – frequency distribution of body temperatures.

$p(T_{\mathrm{e}})$ – frequency distribution of operative temperatures.

W – total fitness integrated over some time interval.

w – fitness.

$w(T_{\mathrm{b}})$ – relationship between fitness with body temperature.

Figure 1

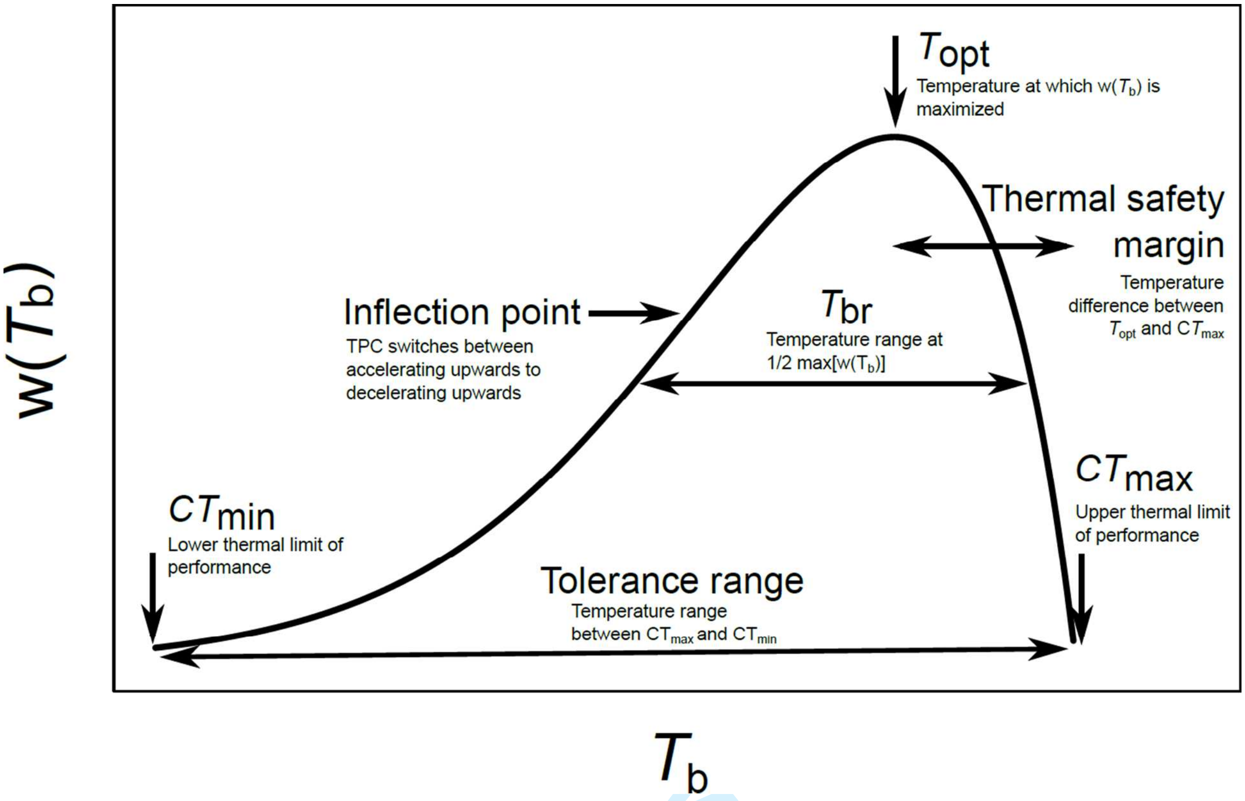

Figure 1. A typical thermal performance curve relating body temperature, T_b to fitness $w(T_b)$, with critical features highlighted (based on Huey & Stevenson 1979). CT_{min} and CT_{max}: Critical thermal minimum and maximum, respectively; T_{opt}: thermal optimum; T_{br}: Thermal breadth. This curve is based on the digestion equation from Stevenson et al. (1985).

Figure 2

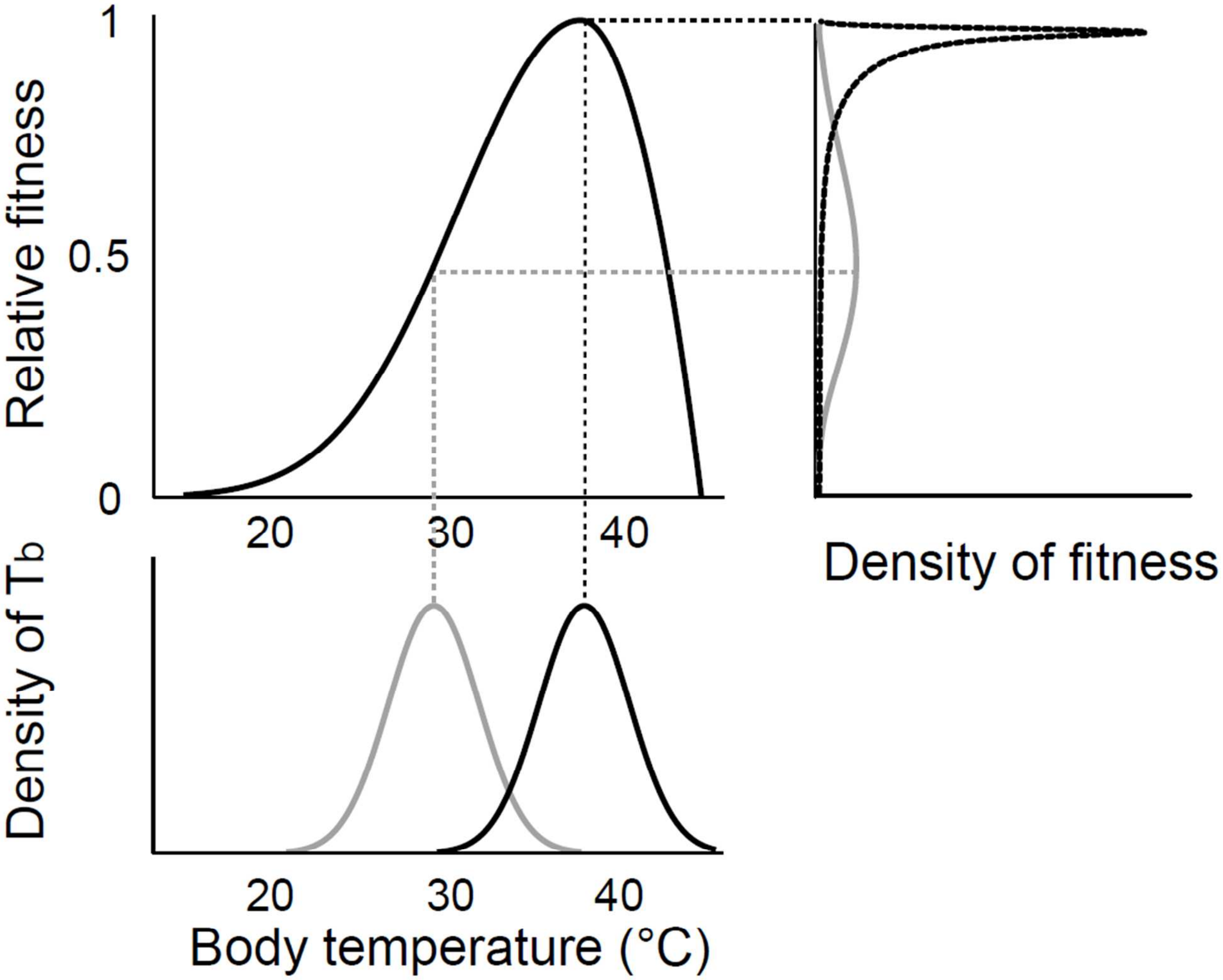

Figure 2. a) An hypothetical *TPC* showing relative fitness (0 to 1) as a function of body temperature (see also Fig. 1). b) Two representative distributions of body temperatures (grey = low T_b, black = high T_b). c) Density of relative fitness for the two T_b distributions in b), calculated from Eqn. 1. The average fitness is much higher for the ectotherm with the higher T_b distribution. Based on Vasseur et al. (2014).

Figure 3

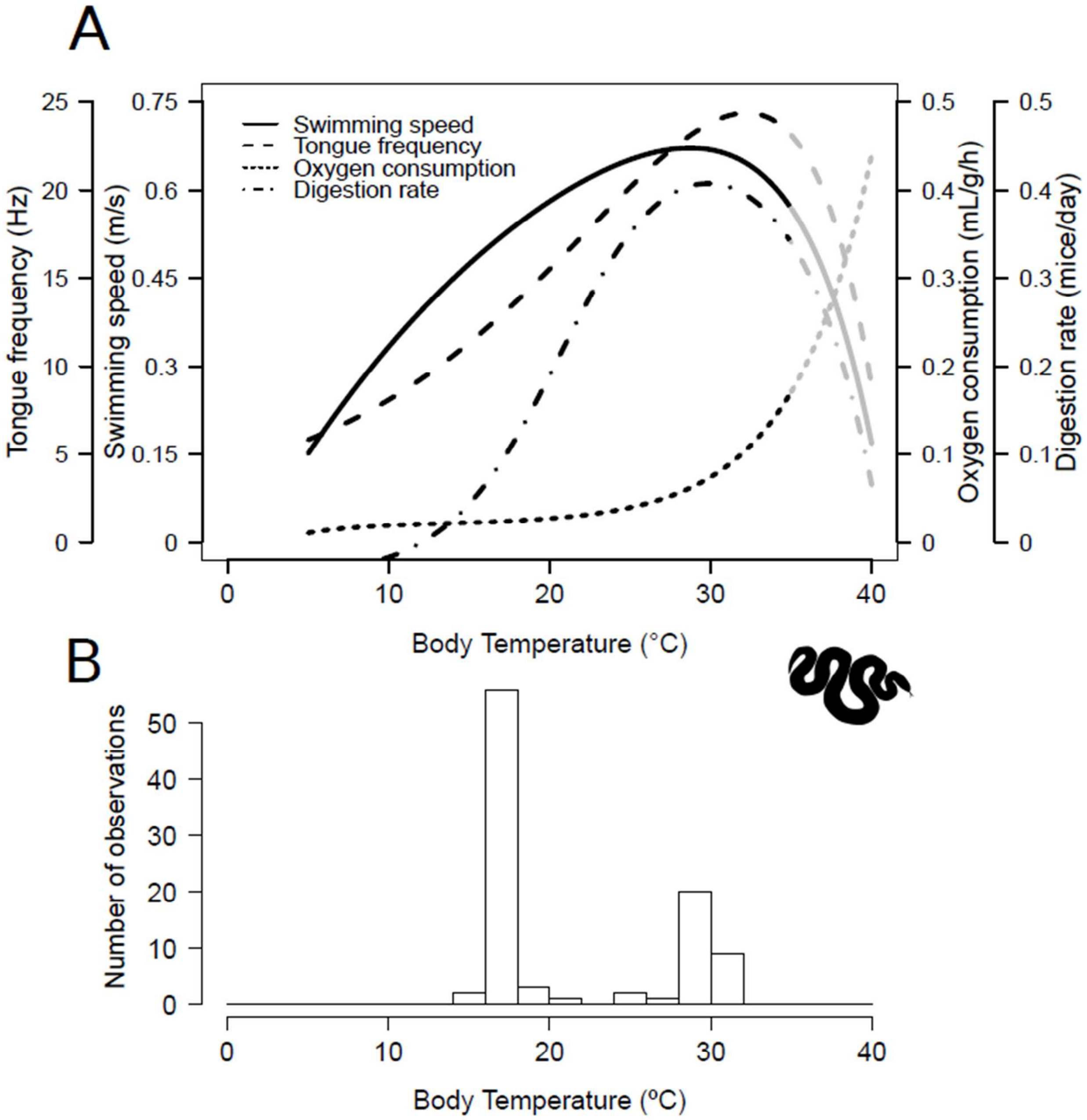

Figure 3. A) Variation in thermal performance curves for four different traits measured in the western garter snake *Thamnophis elegans*. Grey lines indicate parts of the curve that were extrapolated beyond the range of empirical data. B) The distribution of *T. elegans* field body temperatures as measured by radiotelemetry at 15 min intervals over the course of 24 h. Note the significant difference between the distribution of body temperatures and the *TPC*. Data from Stevenson *et al.* (1985).

Figure 4

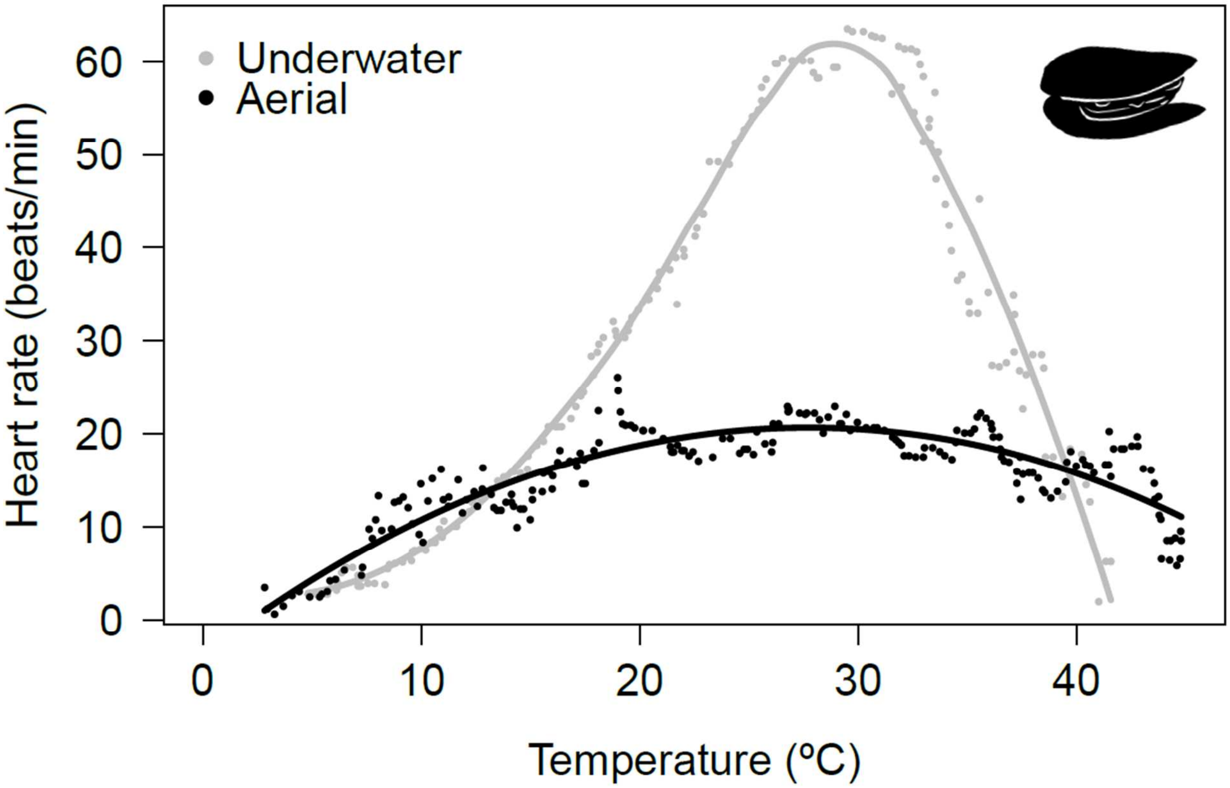

Figure 4. Sensitivity of thermal performance of heart rate to immediate conditions in the brown mussel *Perna perna*. Black lines and points are for emersed mussels, grey lines and points for immersed. Data from Tagliarolo and McQuaid (2015). Lines of best fit are plotted using a locally-weighted polynomial regression implemented by the loess function in R.

Figure 5

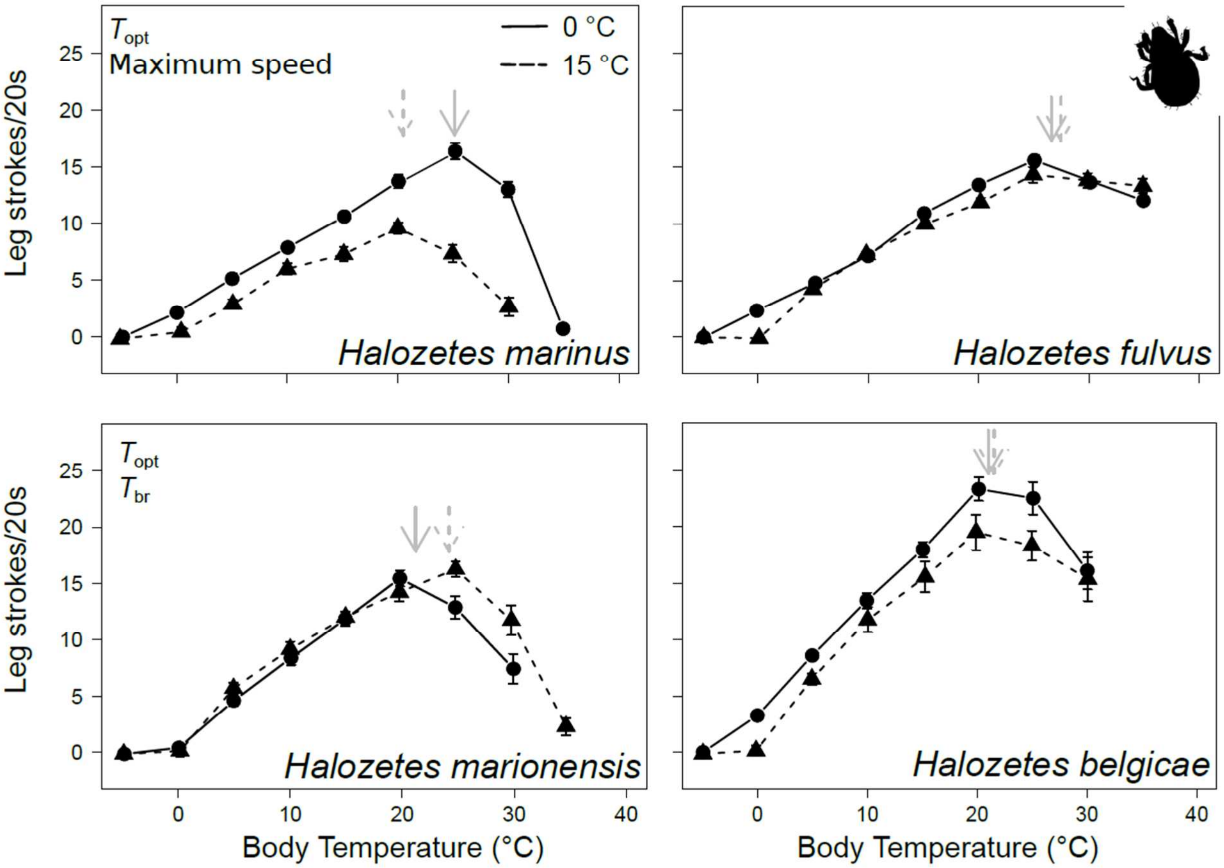

Figure 5. Variation in form and phenotypic plasticity of thermal performance curves of locomotor activity for four congeneric species of sub-Antarctic oribatid mites after acclimation at warm (15 °C, triangles, dashed lines and arrows) and cool (0 °C, circles, solid lines and arrows) temperatures for 7 d. Arrows indicate T_{opt}. Parameters that differ significantly between acclimation treatments are listed in the top left of each plot. Data from Deere and Chown (2006).

Figure 6

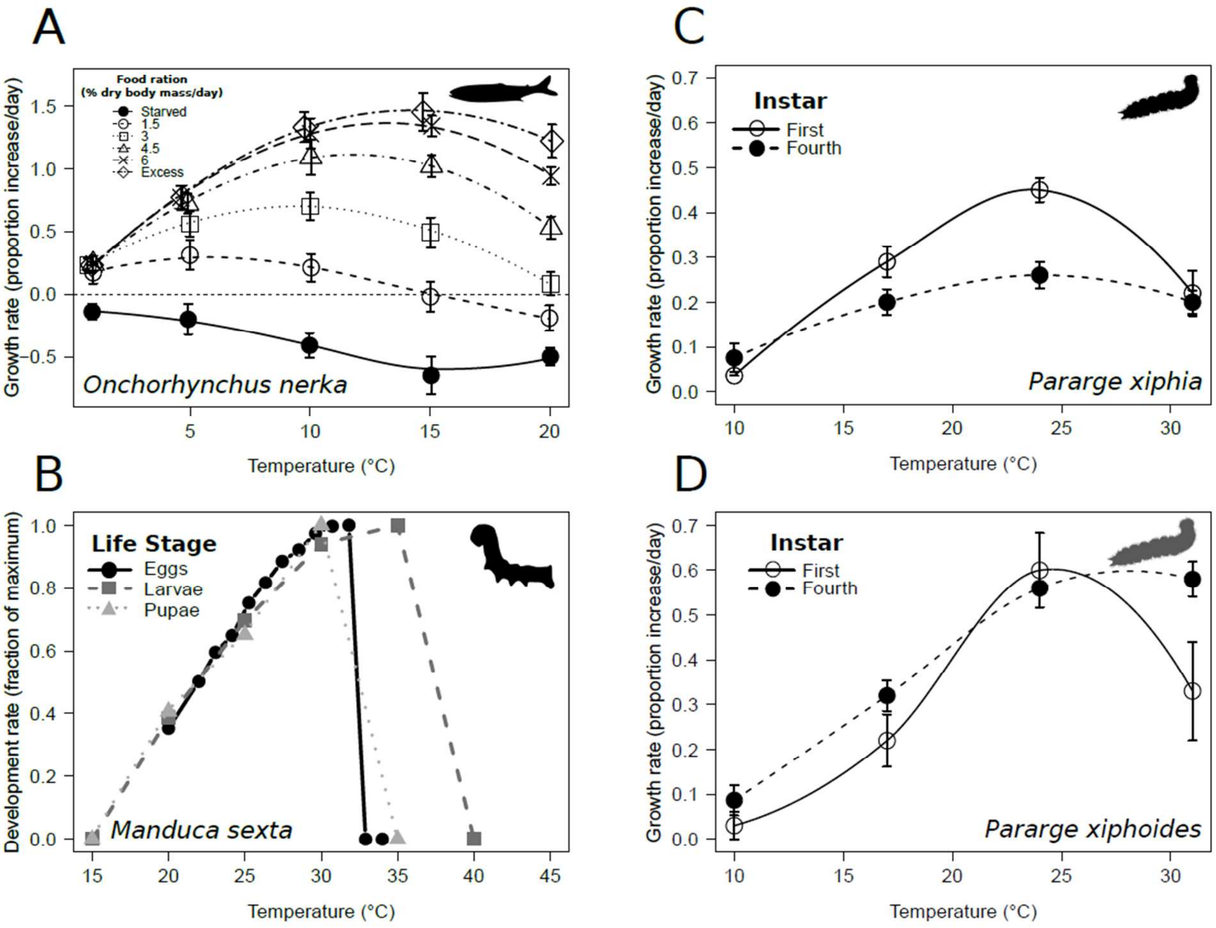

Figure 6. Both nutritional state and ontogenetic stage can affect *TPC*s. (**A**) Varying food ration substantially changes the *TPC* position and magnitude of T_{opt} or growth rate in juvenile sockeye salmon (*Oncorhynchus nerka*). Data from Brett et al. (1969). (**B**) *TPC* shape is determined by developmental stage (eggs, larvae, or pupae) in the sphinx moth *Manduca sexta*. Data from Kingsolver et al. (2011). (**C, D**) Species × development interaction in *TPC*s for growth rate of nymphalid caterpillars in the genus *Pararge*. The among-species shift likely reflects behavioral differences: *P. xiphia* becoming increasingly nocturnal and *P. xiphoides* increasingly diurnal over the course of development. Data from Berger et al. (2011).

Figure 7

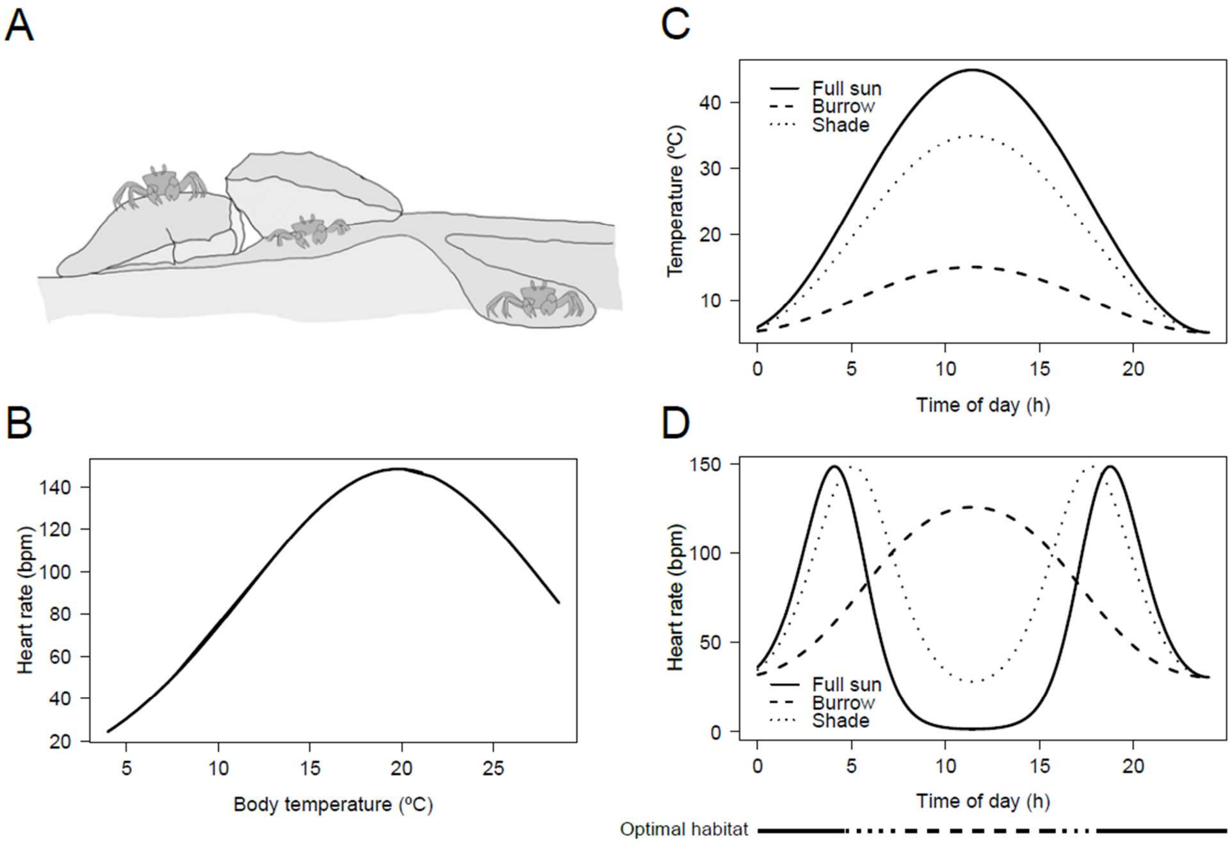

Figure 7. Behavioral thermoregulation can decouple environmental temperature and body temperature. In this example, crabs may choose between full sun, shade, and burrow habitats (**A**). The *TPC* of heart rate in the porcelain crab *Petrolisthes violaceus* (B) is based on data from Gaitán-Espitia et al. (2014). Environmental temperature varies across habitats through the day (**C**), data from Schneider and Helmuth (2007), and so heart rate in *P. violaceus* (modelled from B and C) will depend on which habitat it is occupying at which time of day (**D**), with the optimal habitat (where heart rate is maximized) varying through the course of the day.