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SYMPOSIUM

Thermal Performance Curves, Phenotypic Plasticity, and the Time Scales of Temperature Exposure

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Synopsis Thermal performance curves (TPCs) describe the effects of temperature on biological rate processes. Here, we use examples from our work on common killifish (*Fundulus heteroclitus*) to illustrate some important conceptual issues relating to TPCs in the context of using these curves to predict the responses of organisms to climate change. Phenotypic plasticity has the capacity to alter the shape and position of the TPCs for acute exposures, but these changes can be obscured when rate processes are measured only following chronic exposures. For example, the acute TPC for mitochondrial respiration in killifish is exponential in shape, but this shape changes with acclimation. If respiration rate is measured only at the acclimation temperature, the TPC is linear, concealing the underlying mechanistic complexity at an acute time scale. These issues are particularly problematic when attempting to use TPCs to predict the responses of organisms to temperature change in natural environments. Many TPCs are generated using laboratory exposures to constant temperatures, but temperature fluctuates in the natural environment, and the mechanisms influencing performance at acute and chronic time scales, and the responses of the performance traits at these time scales may be quite different. Unfortunately, our current understanding of the mechanisms underlying the responses of organisms to temperature change is incomplete, particularly with respect to integrating from processes occurring at the level of single proteins up to whole-organism functions across different time scales, which is a challenge for the development of strongly grounded mechanistic models of responses to global climate change.

Introduction

Temperature has profound effects on biological functions at levels of organization from molecules to ecosystems (Hochachka and Somero 2002), and is thus thought to be one of the critical abiotic factors influencing the distribution and abundance of organisms. Two principle aspects of the thermal biology of ectotherms play a central role in shaping distribution patterns: temperature tolerance and temperature-dependent effects on performance. The breadth of the zone of temperature tolerance for a species may vary among populations, life stages, with age, or within a life stage due to phenotypic plasticity (Bowler and Terblanche 2008), but despite these complications, the zone of tolerance of a species is often correlated with aspects of a species' thermal environment in nature (Stillman 2002; Chown et al. 2004, 2010; Clusella-Trullas et al. 2011; Sunday et al. 2011), highlighting the selective importance of this trait. Within the zone of tolerance, temperature also affects performance at a variety of levels of biological organization. Comparisons of temperature-dependent performance across latitudinal and altitudinal thermal clines clearly establish the importance of a variety of temperature-dependent performance traits for setting species' distributions (reviewed in Angilletta 2009). At the whole-organism level, commonly studied performance traits include functions such as fecundity, growth, metabolic rate,

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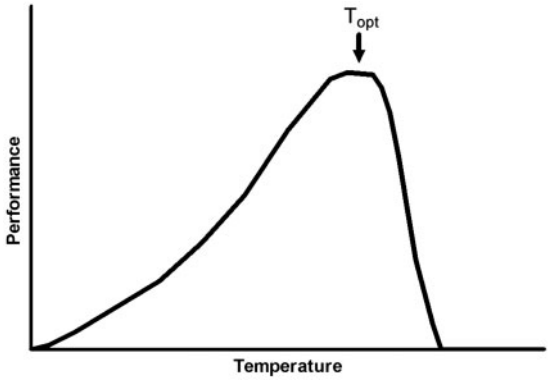

Fig. 1 Hypothetical Thermal Performance Curve (TPC). The temperature at which performance is maximized is termed the $T_{opt.}$ The points at which performance is zero are termed the critical temperatures (T_c, T_{crit}, or CT_{max} and CT_{min}, depending on the author). TPCs can also be used to define the thermal breadth, which is the range of temperatures at which performance meets or exceeds an arbitrary threshold (often 80% of performance at T_{opt}).

and running speed. At the underlying physiological and biochemical levels, performance traits include functions such as heart rate, nerve conduction velocity and enzyme activity. Importantly, performance traits are generally biological rate processes with a time-dependent component (e.g., offspring per lifetime, amount of oxygen consumed per unit time, distance traveled per unit time, or amount of substrate converted to product per unit time).

The effects of temperature on performance traits within the zone of tolerance can be visualized using a thermal performance curve (TPC; Fig. 1). Empirical evidence suggests that TPCs tend to take the same general shape: performance typically increases as temperature increases, reaches a maximum at some intermediate temperature (T_{opt}), and then rapidly decreases (Huey and Stevenson 1979; Huey and Kingsolver 1989, 1993; Angilletta et al. 2002; Angilletta 2009). In principle, adaptive evolution or phenotypic plasticity can modify a TPC by altering its height (through a vertical shift of the curve), the position of the T_{opt} (through a horizontal shift of the curve), and the width (or breadth) of the curve (Huey and Kingsolver 1989; Angilletta et al. 2003; Izem and Kingsolver 2005; Frazier et al. 2006; Angilletta 2009; Kingsolver 2009), as well as by altering the shape of the curve (e.g., linear, exponential, polynomial) in the rising or descending phases, although this last possibility has not often been investigated.

There has been a recent resurgence in interest in TPCs because of their potential utility in helping to predict the responses of populations or species to climate change (Deutsch et al. 2008; Angert et al. 2011; Dell et al. 2011; Huey and Kingsolver 2011). In particular, TPCs can be incorporated into mechanistically based models of responses of organisms to climate change (Helmuth et al. 2005; Kearney 2006; Kearney and Porter 2009; Angert et al. 2011), which may have advantages relative to other approaches (such as climate envelope models; Pearson and Dawson 2003; Guisan and Thuiller 2005) for predicting the effects of climate change on species persistence (Kearney 2006; Buckley et al. 2010; Chown et al. 2010).

The purpose of the current review is to highlight two important issues with respect to TPCs that must be considered when using these data in the context of climate change modeling, and for studies of thermal adaptation more generally. The first issue relates to the time scale of the thermal exposure on the *x*-axis of the TPC. Some models of the effects of climate change use TPCs for the acute effects of temperature (Sitch et al. 2003), whereas other models use TPCs generated following chronic thermal exposure, often to constant temperatures in the laboratory (Deutsch et al. 2008). These types of models thus examine different aspects of thermal biology, and both neglect (at least in part) the complex interplay between instantaneous thermal sensitivity and the ability of organisms to alter their TPCs in response to prior thermal exposure. The second issue we deal with in this review relates to the shape of the TPC itself. The shape of a TPC is usually ascribed to a combination of the thermodynamic effects of temperature on the reaction rates and the destabilizing effects of temperature on intermolecular interactions (Hochachka and Somero 2002). These mechanisms explicitly address the acute effects of temperature at the biochemical level, but the extent to which these mechanisms are strong predictors even at acute time scales, and the degree to which these effects can be generalized to explain the shape of TPCs for longer term exposures and for traits at higher levels of biological organization is not at all clear. Having a strongly grounded mechanistic understanding of the shape of TPCs is important because the shape of the TPC can have a substantial influence on the output of models of the effects of climate change (Wythers et al. 2005). To discuss these issues, we utilize some of our own data on the effects of temperature on the metabolic rate of a small estuarine fish, *Fundulus heteroclitus* (the common killifish), as a framework with which to illustrate the potential problems with the application of TPCs in predicting the effects of climate change.

Acute thermal sensitivity

Metabolic rate is a particularly interesting performance trait to consider in the context of this discussion, in part because theories such as the metabolic theory of ecology (MTE) suggest that metabolic rate is an important component of a synthetic framework that may explain many ecological patterns (Brown et al. 2004), including responses of organisms to climate change (Duarte 2007). Similarly, the theory of oxygen and capacity limited thermal tolerance (OCLTT; Pörtner 2002, 2010) suggests that the effect of temperature on metabolic rate, and specifically on aerobic scope (or the difference between basal and maximal metabolic rate), is an important mechanistic basis for the effects of climate change on the distribution and abundance of species, particularly for fishes and aquatic invertebrates (Pörtner and Knust 2007; Pörtner and Farrell 2008).

We can illustrate the acute effects of temperature on metabolic rate at the biochemical level by considering Fig. 2, which shows the rates of oxygen consumption by liver mitochondria isolated from common killifish, *F. heteroclitus macrolepidotus*, acclimated to constant temperatures of 5, 15, or 25°C in the laboratory and exposed to acute thermal challenge *in vitro* (Fangue et al. 2009a). Oxygen consumption by these mitochondria increases roughly exponentially with acute increases in temperature until the mitochondria are no longer functional (i.e., until the rate of proton leak across the membrane becomes so great that oxygen consumption is a poor predictor of ATP generation, and the mitochondria are said to be uncoupled).

The exponential shape of the curves shown in Fig. 2 is thought to be due to the fundamental thermodynamic effects of temperature on molecular movements. Increases in temperature, and thus in thermal energy, cause the rates of chemical reactions to increase by affecting both the number and energy of collisions between molecules. These effects are summarized by the Arrhenius equation, which can be written as:

$$k = Ae^{-E_a/kT}$$

where k is the rate of a reaction, A is a term called the frequency factor or the pre-exponential factor, E_a is the activation energy of a reaction, k is the Boltzman constant, and T is the temperature in kelvin. Overall, the Arrhenius equation predicts that if E_a does not vary with temperature, then the acute thermal response of a rate process should be exponential in shape up to the T_{opt}.

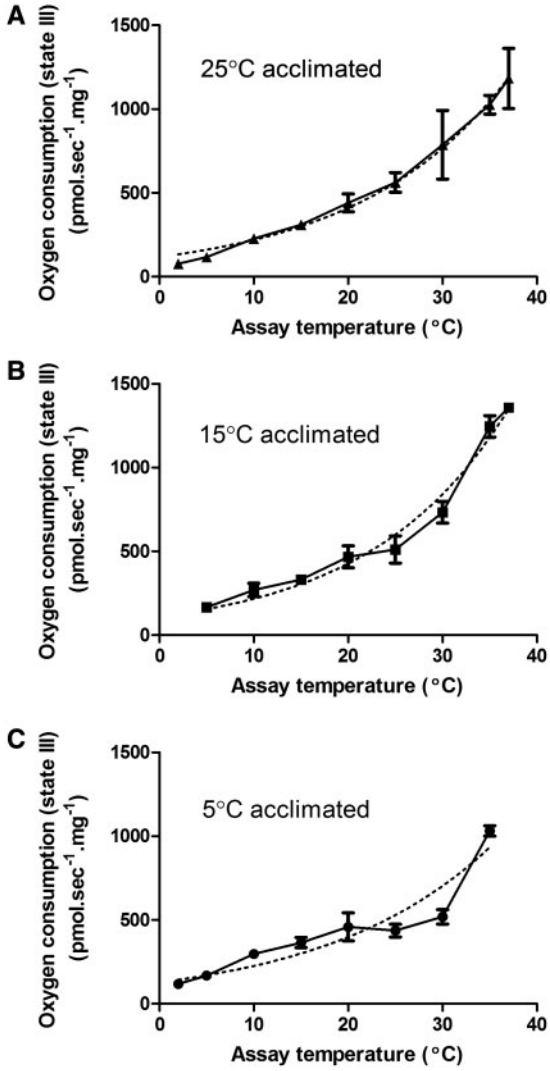

Fig. 2 Maximal consumption of oxygen by killifish mitochondria exposed to acute thermal challenge *in vitro*. (**A**) Liver mitochondria isolated from killifish (*F. heteroclitus macrolepidotus*) acclimated to 25°C. (**B**) Liver mitochondria isolated from killifish acclimated to 15°C. (**C**) Liver mitochondria isolated from killifish acclimated to 5°C. Mitochondria were provided with saturating substrate (pyruvate + malate), oxygen, and ADP to stimulate maximum oxygen consumption. Oxygen consumption is expressed per milligram mitochondrial protein. Data are from Fangue et al. (2009a). Dashed line is the best exponential fit to each dataset; solid line connects the data points.

Arrhenius-like effects can only explain the rising phase of TPCs. The descending phase of the curve is generally ascribed to the destabilizing effects of temperature on the intermolecular interactions. Such biophysical models typically include temperature-dependent irreversible (Sharpe and DeMichele 1977; Schoolfield et al. 1981) or reversible (Peterson et al. 2004; Daniel and Danson 2010) protein denaturation. These models predict that TPCs will not

always follow an exponential shape in their rising phase, and instead predict that TPCs will take a variety of shapes depending on the biophysics of the specific enzyme involved in the reaction. This variety of predicted shapes suggests that models assuming a single (generally exponential) shape for TPCs, such as the metabolic theory of ecology, may not always be correct (Knies and Kingsolver 2010), or indicates that such models lack a strong mechanistic foundation (Clarke 2004; Clarke and Fraser 2004).

Generalizing from phenomena at the level of a single molecule up to higher levels of organization is also problematic. For example, the models above assume that substrate concentrations are saturating for enzyme-catalyzed reactions. However, at low substrate concentration another factor—the Michaelis constant (K_m; concentration of substrate at which the rate of reaction is half of the maximal rate)—becomes important. The K_m of a reaction typically increases with temperature, which would be predicted to alter the shape of the acute TPC. A number of models, largely generated by plant biologists, take this effect into account (reviewed by Atkin and Tjoelker 2003; Davidson et al. 2006; Kruse et al. 2011). These models generally predict that acute TPCs will not be exponential functions in the rising phase, and may take quite complex shapes. Note that the TPCs for the oxygen consumption of killifish mitochondria shown in Fig. 2 were obtained for mitochondria provided with saturating levels of carbon substrate, Adenosine diphosphate (ADP), and oxygen *in vitro*, and are roughly exponential, as predicted by these models (Kruse et al. 2011). In contrast, the TPC for oxygen consumption of 5°C acclimated killifish at the whole-organism level (Fig. 3) takes a somewhat different shape, with a region of low slope at colder temperatures, a pseudo linear region at intermediate temperatures, and a plateau at higher temperatures (Fangue et al. 2009a), indicating that factors other than those evident for isolated mitochondria tested with saturating substrate come into play at higher levels of organization. Similarly, the TPC for aerobically powered swimming in killifish does not take an exponential shape in its rising phase (Fangue et al. 2008), again suggesting a lack of parallelism between TPCs at various levels of biological organization.

The question of whether the combined acute TPCs of cellular networks and complex organismal phenotypes would be expected to be similar to those of relatively simple biochemical models outlined above remains unresolved, in part because of the lack of carefully articulated mechanistic models to connect processes at various levels of biological organization

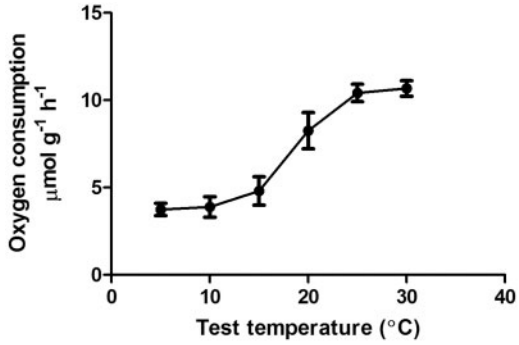

Fig. 3 Whole-organism routine oxygen consumption of killifish (*F. heteroclitus macrolepidotus*) acclimated to 5°C and exposed to an acute increase in temperature. The experiment was performed in a repeated-measures design starting at the acclimation temperature and monitoring oxygen consumption with increasing temperature. As a result, fish were exposed to increasing temperatures for ~4 h prior to measurement at the highest temperature. Data are from Fangue et al. (2009a).

(Clarke 2004; Clarke and Fraser 2004; Clarke and Pörtner 2010). The possibility that TPCs at higher levels of biological organization simply follow the TPC of a single dominant biochemical process is unlikely because modern biochemical theory predicts that control of pathway flux is often distributed among the various components of a metabolic pathway (Fell 1997). Control can also shift from one process to another depending on the conditions under which flux is assessed (e.g., at different temperatures) (Dufour et al. 1996; Atkin and Tjoelker 2003; Chamberlin 2004). In addition, *a priori,* one might expect the mechanisms limiting performance might be different across different thermal ranges, given the very different biophysical constraints operating at, for example, high and low temperatures. Thus, the shape of TPCs is more likely to depend upon the total number of controlling steps and how much the shapes of the underlying TPCs differ, than on the TPC of any one step.

Alternatively, the shapes of TPCs at higher levels of organization could be determined by the emergent properties of complex networks that are not captured at lower levels of organization. For example, recent theoretical work (Ruoff et al. 2007) suggests that it is possible for network processes to be thermally independent even when all of the underlying steps have strong temperature dependence, at least for certain types of network architecture. In addition, *in vivo,* other factors (such as substrate concentrations, membrane fluidity, or other cellular conditions) may vary with temperature, changing the rate of chemical reactions by mechanisms independent of the direct thermodynamic effects on reaction rates or protein denaturation (Atkin and Tjoelker 2003).

Together, these considerations reduce the likelihood that the shape of a TPC is the result of simple thermodynamic effects analogous to those acting on a single protein, particularly at higher levels of organization (Weibel et al. 1996).

Finally, it is also possible that the shapes of TPCs at higher levels of biochemical organization could be influenced by processes only distantly related to underlying biochemical effects. This class of explanation suggests that the shape of TPCs at higher levels of biological organization is the result of various evolutionary tradeoffs among competing processes (Angilletta et al. 2003; Clarke 2004; Clarke and Pörtner 2010). Taken together, all of these considerations indicate that underlying mechanisms can result in a variety of shapes for TPCs at the whole-organism level. This variety of predicted shapes leads to complications in using mechanistically based models to estimate the effects of climate change, as it may not be correct to assume that all processes will be governed by TPCs with similar shapes.

Acute responses to temperature

Because chemical reactions are inherently sensitive to temperature on an acute time scale, the acute TPCs discussed above summarize a type of plasticity that has been called ''nonadaptive'' or ''passive'' phenotypic plasticity (Gotthard and Nylin 1995; Ghalambor et al. 2007; Whitman and Agrawal 2009), which is equivalent to the thermal sensitivity of a process. But organisms can also exhibit what has been termed ''active plasticity'', which requires a specific response by the organism. Note that active plasticity may be beneficial or deleterious (Woods and Harrison 2002), but in all cases it requires a biochemical or physiological response to the environmental stimulus, rather than a passive biophysical effect. In general active plasticity will thus require a sensing mechanism, a transduction system, and a response system. Active plasticity can be very rapid. Figure 4 shows the time course of increases in the levels of mRNA encoding a heat-shock protein in killifish exposed to thermal stress, and demonstrates that changes in mRNA levels can occur within a few minutes, which could then be followed by changes in the amount of protein (Healy et al. 2010). Changes in protein phosphorylation state can occur even more rapidly (Park and Jang 2011). Similarly, blood flow to tissues or ventilation rates can be altered on an almost instantaneous basis.

Complex whole-organism traits can also be altered very rapidly. For example, during a series of

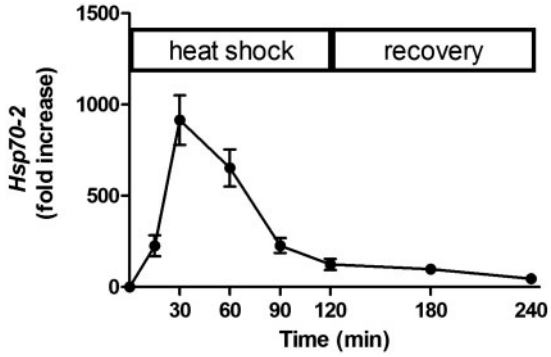

Fig. 4 Fold increases in mRNA levels of heat-shock protein 70 (*hsp70-2*) during and following heat shock in killifish gills. Killifish (*F. heteroclitus macrolepidotus*) acclimated to 20°C were exposed to an acute heat shock of 34°C for 2 h followed by recovery at 20°C for 2 h. Levels of mRNA were determined by real-time quantitative PCR with isoform-specific primers from gill cDNA. Data are from Healy et al. (2010).

experiments on the effects of acclimation temperature on the thermal preference of *F. heteroclitus*, we serendipitously observed some extremely rapid changes in maximum thermal tolerance in response to thermal exposure (Fangue et al. 2009b). In these experiments, killifish were acclimated to temperatures of 5, 15, or 25°C for several weeks. They were then introduced into a thermal-gradient apparatus that provided access to temperatures from ~5 to 35°C, and their position within the gradient was monitored. During preliminary experiments, we found that killifish (of the northern subspecies *F. heteroclitus macrolepidotus*) acclimated to 5°C entered the high-temperature zone of the gradient immediately after they were introduced into the apparatus, causing them to lose equilibrium and be unable to escape these temperatures (i.e., they reached their maximum thermal tolerance). The inability to detect and avoid potentially lethal high temperatures has been observed in other fish species, and is termed ''low thermal responsiveness'' (Meldrim and Gift 1971; Beitinger and Magnuson 1976). Interestingly, killifish of the southern subspecies (*F. heteroclitus heteroclitus*), which have higher maximum thermal tolerance (Fangue et al. 2006), do not exhibit this behavior. The only way in which we could conduct thermal-preference trials on the northern subspecies of killifish was to artificially exclude them from experiencing temperatures above 27°C for the first 1.5 h of the trial, after which we removed the barrier. At that point, these fish could enter regions of high temperature where they had previously lost equilibrium. These observations strongly suggest that killifish undergo some form of physiological acclimation that alters whole-organism

thermal tolerance within a few hours of exposure to moderately high temperatures.

The rapidity with which organisms can respond to changes in temperature makes assessing the extent of passive plasticity in a trait difficult, because from a practical point of view it may be impossible to measure the traits of interest on a time scale that is short enough to entirely avoid physiological adjustments by the organism. For example, assessing performance traits such as oxygen consumption or sustained locomotion often requires 30 min or an hour, and it can be difficult to eliminate the possibility that organismal responses (either beneficial or deleterious) are modifying the trait during the measurement period. Despite these methodological complications, however, we contend that making the distinction between acute sensitivity (passive plasticity) and organismal responses (active plasticity) is an important one from a conceptual and mechanistic perspective when applying TPCs in the context of models predicting the effects of climate change.

Acclimation

Physiologists have long been interested in a particular class of phenotypic plasticity termed acclimation, which is typically defined to include reversible changes in physiological phenotypes as a result of environmental exposures in the time range of days to months. Note, however, that there is a fuzzy boundary between acute physiological responses, which can occur within minutes or hours (e.g., the heat-shock response), and more typical acclimation responses on a longer time scale. In addition, the degree of reversibility of all of these responses may vary, with prior thermal exposure having the potential to cause long-term effects on phenotype (Whitman 2009). If we return to Fig. 2, it is apparent that the TPCs for the acute effects of temperature on mitochondrial oxygen consumption differ when compared between mitochondria from fish held at different acclimation temperatures (Fig. 2A–C). Both the exponent (slope) and the intercept of the exponential curves fitted to the data differ, and there is also a slight movement of the curve along the *x*-axis; mitochondria from killifish acclimated at 5°C could not be assayed at temperatures greater than 35°C, while those from killifish acclimated to warmer temperatures could be assayed at temperatures up to 37°C. In addition, the data from the 25°C acclimation group conform fairly well to an exponential curve, but there is increasing deviation from a simple exponential curve and an increase in obvious breakpoints with decreasing acclimation temperature. Overall, the data shown in Fig. 2 indicate that one of the important effects of acclimation on oxygen consumption by killifish mitochondria is to change the nature of their response to acute temperature challenge.

The idea that thermal acclimation of biological rate processes (which are affected by temperature on an instantaneous basis) is likely to be due to changes in the position and shape of the TPC on an acute time scale has long been appreciated (Precht 1949; Prosser 1958). However, many studies on biological rate processes fail to consider this shorter temporal scale, and instead simply examine metabolic rate at the acclimation temperature across various treatments. In fact, it has been argued that this is the most biologically relevant way to assess performance if you wish to estimate selection on TPCs, as animals are most likely to be exposed to temperatures close to the temperature to which they are acclimatized in nature. For example, one of the most convincing empirical examinations of the hypothesis of oxygen and capacity limited thermal tolerance is an examination of variation in the TPCs for aerobic scope in different stocks of sockeye salmon (Eliason et al. 2011), which found a strong correlation between the T_{opt} for aerobic scope, a variety of underlying physiological traits, and the historical mean river temperature at the time of migration. This study utilized fish acclimated for 1 week to the temperature at which they were collected, and physiological rate processes were tested only at this acclimation temperature.

The potential challenges associated with examining rate processes at the temperature of acclimation can be seen if we replot the data on the oxygen consumption of killifish mitochondria from Fig. 2 to show oxygen consumption at the acclimation temperature (Fig. 5). In this case, oxygen consumption increases linearly with acclimation temperature, unlike the exponential increases seen in the acute TPCs (Fig. 2). Thus, in cases where an organism has the capacity to alter the acute TPC by acclimation, considering only the TPC on an acclimated time scale (as in Fig. 5) will conceal the underlying mechanistic complexity. This observation highlights a critical property of TPCs. As pointed out by David et al. (2003), most studies of phenotypic plasticity assume a null hypothesis of no passive plasticity at the acute time scale, but for TPCs, the null hypothesis is the presence of passive plasticity (sensitivity to temperature) at the acute time scale, making the interpretation of plasticity from TPCs on longer time scales of temperature exposure more complicated than for many other traits.

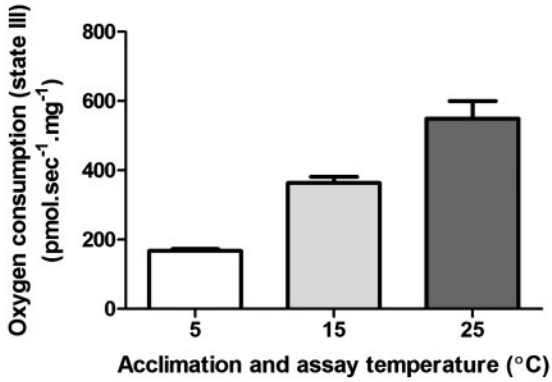

Fig. 5 Maximal *in vitro* (state III) consumption of oxygen by killifish mitochondria at the acclimation temperature. Liver mitochondria were isolated from killifish (*F. heteroclitus macrolepidotus*) acclimated to three different temperatures and were tested *in vitro* at their acclimation temperature. Mitochondria were provided with saturating substrate (pyruvate + malate), oxygen, and ADP to stimulate maximum oxygen consumption. Oxygen consumption is expressed per mg mitochondrial protein. Data are from Fangue et al. (2009a).

The interactions between passive plasticity and active plasticity have prompted a certain degree of confusion with respect to terminology. The extent of phenotypic plasticity for a trait is usually visualized using a reaction norm (Woltereck 1909), which is simply a graph of the values of a trait of interest against values of an environmental variable. The slope of a reaction norm summarizes the extent of plasticity in the trait. When the slope of the reaction norm is horizontal, the trait does not vary with the environment (i.e., it lacks plasticity), whereas when the slope of the reaction norm is steep, the trait varies greatly as the environment changes (i.e., it has high plasticity). From this description, the similarities between TPCs and reaction norms are clear; TPCs are simply reaction norms for a particular class of trait (biological rate processes). The complex nonlinear shapes of TPCs make interpretation of the slope somewhat more difficult, but the real difficulties arise because of the presence of passive plasticity at an acute time scale for TPCs.

Imagine, for example, a hypothetical reaction norm showing the effects of acclimation temperature on metabolic rate measured at the acclimation temperature (similar to the presentation shown in Fig. 5). In a case where this graph has zero slope, we would conclude that there is no plasticity in this trait. However, recall that there is substantial passive plasticity in metabolic rate at the acute time scale of temperature exposure. Thus, the only way to achieve a horizontal slope on the acclimated reaction norm is for active plasticity to have caused physiological changes that maintain the acclimated metabolic rate constant with increasing temperature. This leads to the apparently absurd conclusion that the only way to achieve a reaction norm demonstrating a lack of plasticity is for the organism to exhibit substantial plasticity. Of course, this logical knot can be untied by recalling that two different types of plasticity acting at two different time scales are being considered here. To avoid confusion, we would prefer that the term plasticity only be used to describe an active response by the organism, but we acknowledge that this is not the generally accepted usage.

We are not the first to point out the challenges of dealing with environmental effects acting at multiple time scales (Fry 1971; Pigliucci 2001; Chown and Terblanche 2007). For example, Kingsolver et al. (2004) suggested that graphs showing effects of temperature at time scales relevant to acclimation should only be thought of as reaction norms when they take into account the effects of changes in the shape of the curves at acute time scales. Chown and Terblanche (2007) made a similar proposal, suggesting that "where variation in performance curves is being assessed, the use of the term reaction norm should be restricted to the response of the curves rather than being meant to imply the curves too". In this context, we suggest that it might be useful to develop (or encourage the use of) alternatives to typical TPCs that help to keep this potential complexity at the forefront of thinking. At least for traits where it is possible to measure the acute TPC, it would almost certainly be useful to utilize three dimensional plots, with acclimation temperature on one axis and acutely experienced temperature on another. These distinctions can be important, because failing to properly distinguish between passive plasticity and active plasticity can have critical consequences for the development of mechanistically based models of the impacts of climate change (Wythers et al. 2005).

Effects at longer time scales

Adding further complexity to the issue of time scales of thermal exposures, plasticity can also act at longer time scales. For example, much of the early work on phenotypic plasticity focused on a specific kind of plasticity that can be termed developmental plasticity. In this case, the environmental conditions experienced during development result in a switch between alternative fixed phenotypes in the adult organism (Kinne 1962; Whitman and Agrawal 2009). Plasticity may also act at very long time scales, as the environment experienced by a parent has been shown to affect the phenotype of its offspring, even

across multiple generations, in a phenomenon that has been termed transgenerational plasticity (for examples, from plants, see Dyer et al. 2010; Pías et al. 2010). There is also the potential for interactions among plasticity acting at various scales, for example, developmental plasticity and acclimatory plasticity have been shown to interact to influence both thermal tolerance limits and TPCs (Schaefer and Ryan 2006; Terblanche and Chown 2006; Koumoundouros et al. 2009).

Natural selection can also act on TPCs, influencing their shape and position, as well as the extent of active phenotypic plasticity. Although evolution by natural selection has often been considered to act too slowly to be a response to anthropogenic climate change (Visser 2008; although see Hendry and Kinnison 1999; Gingerich 2009) substantial evidence from experimental evolution studies in the laboratory suggests that thermally relevant traits, including maximum and minimum thermal tolerance, thermal optimum, and the shape of TPCs can change rapidly in response to selection (Knies et al. 2006; Angilletta et al. 2010). Many of these studies, however, use exposures to constant extreme temperatures as the selective agent, so their relevance to the effects of anthropogenic climate change remains unclear. Recently, we participated in a study of the rates of evolution in thermal tolerance in stickleback (*Gasterosteus aculeatus*) that utilized natural environmental exposures as the selective agent (Barrett et al. 2008). In British Columbia, natural populations of stickleback that are resident in freshwater habitats have greater tolerance of cold temperatures than do the ancestral marine populations (Barrett et al. 2011). To examine the rates of evolution in cold tolerance in this species, we stocked large freshwater experimental ponds with wild-caught stickleback of marine origin, which were then allowed to breed naturally in the ponds for several years. By the F3 generation, cold tolerance had improved such that it was significantly different from that of the ancestral populations, and not different from that of naturally evolved freshwater populations, even when all the fish were maintained under common conditions in the laboratory (Barrett et al. 2011). There were substantial shifts in gene frequencies in these populations across generations (Barrett et al. 2008), consistent with the action of adaptive evolution. However, because offspring of individuals from the pond experiments have not yet been reared under common environmental conditions in the laboratory through multiple generations, we cannot eliminate the possibility that developmental plasticity rather than adaptive evolution could account for the observed improvement in cold tolerance in these populations. Whatever the mechanism, however, this experiment demonstrates that exposure to realistic thermal conditions can drive rapid changes in thermally relevant phenotypes.

TPCs and the effects of climate change

Anthropogenic climate change is expected to be associated both with changes in mean conditions and an increase in the frequency of extreme events (IPCC 2007), but the greatest attention has been paid to the effects of changes in mean climate. In contrast, studies in a variety of species have suggested that climatic extremes may be important factors shaping spatial distributions (Stenseth et al. 2002; Sinclair et al. 2003; Helmuth et al. 2006; Harley and Paine 2009; Mulholland et al. 2009; Zimmermann et al. 2009), suggesting that closer attention should be paid to the effects of environmental variability when making predictions about the potential impacts of global climate change. For example, meta-analysis of data for squamate reptiles indicates that environmental temperature variability is a better predictor of preferred temperature and maximum thermal tolerance than is mean habitat temperature (Clusella-Trullas et al. 2011). Thus, incorporating analyses of acute thermal sensitivity into models of the effects of climate change is likely to be critical. In fact, Clusella-Trullas et al. (2011) conclude that models including thermal variation result in altered conclusions about the relative susceptibility of reptiles to climate warming, and suggest that reptiles at mid-latitudes may be at particular risk, rather than those at tropical latitudes as suggested by previous studies (Deutsch et al. 2008).

Since phenotypic plasticity and evolutionary adaptation both have the capacity to alter the position, height, breadth, and shape of acute TPCs, considering only the shape of the acute TPC is also likely to be a mistake when attempting to develop models of the effects of climate change. For example, Wythers et al. (2005) show that vegetation models using only a static acute TPC can yield inaccurate predictions, and instead suggest that models allowing thermal acclimation to change both respiration rate at the acclimation temperature and the shape of the acute response to temperature are more accurate. In this context, there is a clear need for additional theory development with respect to the interplay between environmental variation and mean environmental temperature in shaping TPCs (Boyce et al. 2006; Morris et al. 2008; Reed et al. 2010), and in

predicting the responses of organisms to global climate change (Hoffmann and Sgrò 2011).

Conclusions and perspectives

The purpose of the present review was to point out some important issues associated with understanding and interpreting TPCs, particularly in the context of applying these data to models that make predictions about the responses of organisms to climate change. We focused on: (1) the importance of incorporating the time domain of temperature exposure into these curves, and how that influences the ways in which we think about plasticity in performance traits, and (2) the potential fundamental problems with extrapolating from simple chemical thermodynamics (Arrhenius-like effects) to the complex multistep processes that underlie organismal performance, and how this makes firms conclusions about the likely shapes of TPCs difficult. Finally, bringing both of these ideas together, we emphasized the importance of considering both mean temperature and thermal variability when making predictions about the likely effects of climate change, since traits such as lifetime fecundity represent the integration of thermal exposures across long time scales in which both mean temperature and thermal variability may be critical. Equally important, but beyond the scope of this review, is the likelihood of interactions between temperature and other abiotic and biotic environmental factors that can alter the relationship between temperature and performance, affecting both the TPC and its evolutionary trajectory (Agrawal 2001; Pörtner and Farrell 2008; Harmon et al. 2009; Lavergne et al. 2010). Ultimately, all of these factors are likely to play a role in determining the responses of organisms to climate change. Developing a clearer understanding of the mechanisms via which the environment affects organisms at all levels of organization and across time scales should aid in the development of strongly grounded mechanistic models of potential responses to climate change.

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