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REVIEW

The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment

Patricia M. Schulte*

ABSTRACT

Because of its profound effects on the rates of biological processes such as aerobic metabolism, environmental temperature plays an important role in shaping the distribution and abundance of species. As temperature increases, the rate of metabolism increases and then rapidly declines at higher temperatures – a response that can be described using a thermal performance curve (TPC). Although the shape of the TPC for aerobic metabolism is often attributed to the competing effects of thermodynamics, which can be described using the Arrhenius equation, and the effects of temperature on protein stability, this account represents an over-simplification of the factors acting even at the level of single proteins. In addition, it cannot adequately account for the effects of temperature on complex multistep processes, such as aerobic metabolism, that rely on mechanisms acting across multiple levels of biological organization. The purpose of this review is to explore our current understanding of the factors that shape the TPC for aerobic metabolism in response to acute changes in temperature, and to highlight areas where this understanding is weak or insufficient. Developing a more strongly grounded mechanistic model to account for the shape of the TPC for aerobic metabolism is crucial because these TPCs are the foundation of several recent attempts to predict the responses of species to climate change, including the metabolic theory of ecology and the hypothesis of oxygen and capacity-limited thermal tolerance.

KEY WORDS: Aerobic metabolism, Metabolic rate, Aerobic scope, Temperature, Adaptation, Acclimation, Climate change, Thermal performance curve

Introduction

Temperature has profound effects on chemical and biochemical reactions; thus, understanding the mechanisms that organisms use to cope with thermal change has been a focus of the field of biochemical adaptation since its inception (Hochachka, 1965, 1967; Somero et al., 1968; Somero and Hochachka, 1969, 1971; Hochachka and Somero, 1968, 1973, 2002). Over the past 50 years, significant progress has been made in understanding the biochemical basis of thermal adaptation (Cossins and Bowler, 1987; Angilletta, 2009), particularly at the level of individual proteins (Somero, 2004; Fields et al., 2015). However, many questions remain unanswered, even at the biochemical level, and we still lack a full mechanistic understanding of the effects of temperature on biological processes across levels of organization and the suite of adaptations that organisms use to cope with these effects (Somero, 2012). These questions are increasingly critical because human-induced climate change is altering patterns of mean and extreme temperatures across the globe, resulting in changes in the biogeographic distributions of species (Parmesan and Yohe, 2003; Perry et al., 2005; Parmesan, 2006).

The simplest way to describe the effects of temperature on the rate of a biochemical, physiological, or behavioral process is to generate a thermal performance curve (TPC, Fig. 1) (Schulte et al., 2011). Many studies have documented the shapes of TPCs across levels of biological organization and taxa. Meta-analysis of these data (Dell et al., 2013) indicates that TPCs tend to be unimodal and left skewed with three distinct regions (Dell et al., 2011, 2013): (1) a rising phase as temperature increases; (2) a plateau phase that encompasses the thermal optimum (T_{opt}) for the trait; and (3) a steep falling phase at higher temperatures.

Within this commonality, however, there is an enormous amount of variation in the shape of TPCs. This variation is, in part, the result of adaptive differences among taxa, and can also be caused by neutral variation and various types of plasticity, including epigenetic effects, developmental plasticity and acclimation (Schulte et al., 2011), as well as by methodological issues such as the rate of temperature change imposed during the experimental determination of the TPC (Cossins and Bowler, 1987).

At a mechanistic level, the rising phase of the TPC is often described as being caused by thermodynamic effects, which can be described using the Arrhenius equation (Arrhenius, 1915):

$$k = A\mathrm{e}^{-\frac{E_\mathrm{a}}{RT}}. \quad (1)$$

Where k is the rate of a reaction, A is the pre-exponential factor (which is constant at biologically relevant temperatures), E_a is the activation energy of the reaction, R is the gas constant and T is the temperature in kelvin. The Arrhenius equation predicts that the rising phase of a TPC should be exponential in shape if simple thermodynamic effects dominate.

Natural log transformation of the Arrhenius equation results in the following:

$$\ln k = -\frac{E_\mathrm{a}}{RT} + \ln A. \quad (2)$$

If one plots ln k against $1/T$ in kelvin (in an Arrhenius plot), a straight line will be obtained with slope equal to $-E_\mathrm{a}R^{-1}$ up to temperatures just below the T_{opt}, if the process is following the Arrhenius equation. As the TPC reaches the T_{opt}, the slope of the Arrhenius plot should have a significant discontinuity, at a point called the Arrhenius breakpoint temperature (ABT). If more than one ABT is present, or the data show a non-linear relationship with

Department of Zoology, 6270 University Blvd, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4.

*Author for correspondence (pschulte@zoology.ubc.ca)

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List of abbreviations and symbols

ABT	Arrhenius breakpoint temperature
C_P	heat capacity
CT_{max}	critical thermal maximum
k_{cat}	catalytic rate constant
k_m	Michaelis constant
MR	metabolic rate
MMR	maximum metabolic rate
MTE	metabolic theory of ecology
OCLTT	oxygen and capacity limited thermal tolerance
RMR	routine metabolic rate
T_{opt}	optimum temperature
TPC	thermal performance curve
V_{max}	maximal activity
$\Delta G^{\ddagger}$	Gibbs energy of activation

temperature, then the TPC is not following simple Arrhenius predictions (Cossins and Bowler, 1987).

Although the Arrhenius equation may be used to account for the shape of the rising phase of a TPC, it cannot explain the most obvious feature of TPCs for biological processes: the presence of a maximum (at the T_{opt}) followed by a steep decline in rate. This decline is typically attributed to denaturation of proteins at high temperatures. However, even for individual proteins this account represents an oversimplification of the processes that shape TPCs, and it is unclear whether these factors are sufficient when considering complex biological traits caused by the interaction of many proteins and processes at multiple levels of biological organization (Prosser, 1973; Cossins and Bowler, 1987; Knies and Kingsolver, 2010).

Although our understanding of the underlying biochemical and physiological mechanisms that shape TPCs is incomplete, empirical data on TPC shapes are currently being used to develop predictive models about the responses of species, populations and communities to climate change (e.g. see Kordas et al., 2011; Amarasekare and Savage, 2012; Dell et al., 2014; Gilbert et al., 2014). Developing these empirically based models into true cause-and-effect mechanistic models (Helmuth et al., 2005) will require insight into the proximate and ultimate causes of variation in the shape of TPCs. Therefore, the purpose of this review is to examine our current understanding of the underlying biochemical and physiological mechanisms that shape TPCs for aerobic metabolism in response to acute changes in temperature, to provide perspectives on the mechanisms of physiological plasticity and evolutionary adaptation that organisms could use to cope with these effects.

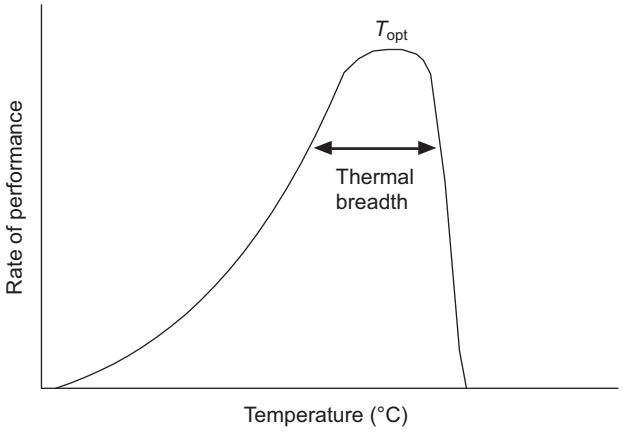

Fig. 1. A thermal performance curve (TPC). TPCs summarize the effects of temperature on the rates of biological processes. Critical features of a TPC are the slope of the increase in rate with temperature, the thermal optimum for the process (T_{opt}) and the thermal breadth, which is the range of temperatures over which the rate of the process is maximized.

Why focus on aerobic metabolism?

Most biochemical reactions are profoundly affected by temperature, but there are several reasons to focus on aerobic metabolism when thinking about the role that temperature plays in shaping the distribution and abundance of species. Aerobic metabolism is so fundamental to animals that it has been termed the 'fire of life' (Kleiber, 1961). Most animals are dependent on the presence of oxygen for at least some parts of their life, and even animals that have evolved strategies for living in anoxic habitats are usually only facultative anaerobes (Tielens et al., 2002; Mentel and Martin, 2010), with complete anaerobes being rare exceptions to this rule (Kristensen, 2002; Danovaro et al., 2010; Mentel and Martin, 2010). Like most biochemical reactions, the reactions of aerobic metabolism are directly influenced by environmental temperature (Hochachka and Somero, 2002), so organisms must have mechanisms to cope with these effects to preserve energy generation across temperatures. Thus, a key reason for focusing on aerobic metabolism is its importance in supplying the energy that is needed for animal life.

A second important reason for focusing on aerobic metabolism is that recent theoretical syntheses have focused on metabolic traits as key players in the responses of organisms to climate change. In particular, both the metabolic theory of ecology (MTE) (Brown et al., 2004) and the hypothesis of oxygen and capacity limited thermal tolerance (OCLTT) (Pörtner, 2001, 2002a,b, 2010; Pörtner and Knust, 2007; Claireaux and Lefrançois, 2007; Pörtner and Farrell, 2008) place the thermal dependence of metabolic rate processes at the center of their syntheses. Thus, understanding the mechanistic basis of the thermal dependence of aerobic metabolism is essential for assessing the validity of these theories.

The MTE is built around the idea that metabolism provides a mechanistic basis for a central unifying theory of ecology (Brown et al., 2012), and it has been widely adopted by ecologists interested in predicting the responses of species to climate change (Whitefield, 2004; Vasseur and McCann, 2005; O'Connor et al., 2009, 2011; Dillon et al., 2010). The MTE derives from earlier work (West et al., 1997; Gillooly et al., 2001) that attempted to provide a mechanistic basis for the prediction of metabolic rate, taking into account the effects of body size and temperature. The MTE extended these ideas across levels of biological organization from that of the individual to that of populations, communities and ecosystems.

Many of the underlying mechanistic aspects of the MTE, which were based on the properties of fractal networks, have been criticized (Clarke, 2004, 2006; Clarke and Fraser, 2004; Cyr and Walker, 2004; O'Connor et al., 2007; Martínez del Rio, 2008) and as a result, the MTE might more accurately be viewed as an empirical rather than a mechanistic theory (Martínez del Rio, 2008; Price et al., 2012). In particular, the MTE makes the assumption that biological processes will conform to the predictions of the Arrhenius equation, with an exponential rising phase, and goes on from this assumption to make predictions about the responses of species to climate change. One of the purposes of this review is to ask whether this assumption is warranted.

The OCLTT hypothesis takes a different view of the role of aerobic processes in determining the responses of organisms to climate change, while still emphasizing their importance. Rather than looking at metabolic rate, per se, the OCLTT focuses on aerobic scope, or the difference between basal and maximal oxygen consumption, which represents the energy that an organism has

available to perform critical functions such as growth, locomotion and reproduction (Pörtner, 2001, 2002a,b; Pörtner and Knust, 2007; Claireaux and Lefrançois, 2007; Pörtner and Farrell, 2008). This theory parallels the early work of Fred Fry and his students (Fry, 1947; Fry and Hart, 1948; Brett, 1971), who suggested that limitations on aerobic scope limit the ability of an organism to perform various ecological functions. The OCLTT, which also links aerobic scope to whole-organism performance and fitness, provided additional mechanistic underpinnings to this idea by considering the various underlying factors that may come into play at different points along the aerobic scope curve.

A final key reason for focusing on aerobic metabolism comes from comparative and evolutionary studies of variation in aerobic metabolic traits. For example, multiple studies have documented correlations between aerobic metabolic functions and gradients of latitude and altitude (Angilletta et al., 2002; Fontanillas et al., 2005; Pörtner, 2006; Hassanin et al., 2009; Cheviron and Brumfield, 2009; Scott et al., 2011; Toews et al., 2014; Stager et al., 2014). Similarly, many studies have demonstrated that variation in aerobic metabolism measured at the tissue and whole-organism levels are associated with differences in performance and fitness, or correlates of fitness (Pörtner and Knust, 2007; Farrell et al., 2008; Eliason et al., 2011; Zera, 2011; White et al., 2012; Rauhamäki et al., 2014). Also, genetic variation at the levels of the mitochondrion has been associated with differences in fitness (Rand, 2001; Ballard and Whitlock, 2004; Blier et al., 2013). Together, these data strongly suggest that aerobic metabolism is under strong environmental selection and is likely to be important in shaping the responses of species to climate change.

Effects of temperature at the level of single proteins

Because of the complexity of the effects of temperature on processes at the cellular and organismal levels, theories such as the MTE typically make the assumption that TPCs at higher levels of biological organization are shaped by the effects of temperature on a key underlying process at the biochemical level. However, even at the protein level, much remains to be learned about the mechanisms that shape TPCs. The classical model to account for the shape of the TPCs for individual proteins ascribes the shape to Arrhenius effects as temperature increases, combined with protein denaturation at high temperatures. One of the main challenges with this model is that denaturation is a time-dependent process; the longer an enzyme spends at high temperature, the more likely it is to be denatured, and since denaturation is irreversible, longer assay times result in greater denaturation and a lower apparent T_{opt}. Another challenge to the classical model is that the observed T_{opt} of a protein can be well below the denaturation temperature for the protein, at least for some enzymes (Feller and Gerday, 2003). For example, consider the myofibrillar ATPase of the killifish *Fundulus heteroclitus*, which has an Arrhenius break temperature at around 12°C (Sidell et al., 1983), but does not denature until ∼40°C.

There are two related models that have been suggested to account for the deficiencies in the classical model. The 'equilibrium model' proposes that enzymes go through a reversibly inactivated form prior to denaturing (Petersen et al., 2004; Daniel et al., 2010; Daniel and Danson, 2010). The increase in the proportion of the reversibly inactivated form as temperature increases gradually negates the increase in reaction rate caused by thermodynamic effects, resulting in a decline in enzyme activity and the characteristic shape of the TPC. The Sharpe–Schoolfield model (Sharpe and DeMichele, 1977; Schoolfield et al., 1981) is similar, but postulates that reversibly inactivated forms occur at both high and low temperatures. Both of these models provide better fits to the observed data compared with the classical model. They are also consistent with suggestions that reversibly denatured intermediates could account for the stabilizing effects of osmolytes at high temperatures (Winzor et al., 1992; Hall et al., 1995; Winzor and Jackson, 2006).

However, both the equilibrium and Sharpe–Schoolfield models may represent over-simplifications of the highly dynamic structures of proteins (Shukla et al., 2015). Rather than occurring in a small number of conformations (e.g. active versus reversibly or irreversibly denatured), recent evidence from biophysics suggests that proteins occur as a population of molecules with a large number of conformations, termed microstates that may differ in catalytic activity or substrate binding (Henzler-Wildman and Kern, 2007; Wrabl et al., 2011). Proteins can switch between these states at various time scales and the probability of switching changes with temperature. Thus, rather than modeling the effects of temperature as a change in the proportion of 'native' and reversibly or irreversibly 'denatured' proteins, it is more likely that changes in temperature cause shifts in the probability landscape connecting many functionally distinct microstates. Until these ideas are fully integrated into our understanding of the effects of temperature on protein function, we will not have achieved a fully mechanistic description of the shape of the TPC at the level of even a single enzyme.

Another model to account for the effects of temperature on protein activity, the macromolecular rate theory (Hobbs et al., 2013), derives from the fundamentals of statistical thermodynamics, and specifically from transition state theory (Eyring, 1935). It is very different from the other models because it does not require enzyme denaturation to account for the shapes of the TPCs for individual proteins (Schipper et al., 2014). To understand the theory, it is necessary to think about the steps via which an enzyme catalyzes the conversion from substrate to product. During this conversion, the substrate molecule passes through a transition state that has a higher free energy than that of either the substrate or the product. The difference in free energy between the initial state and the transition state is termed the Gibbs energy of activation ($\Delta G^{\ddagger}$). An enzyme speeds up reaction rate by stabilizing the transition state, and thus reducing the free energy change associated with transition state formation, which reduces the activation energy barrier ($\Delta G^{\ddagger}$). One of the central assumptions of the transition state theory is that $\Delta G^{\ddagger}$ is independent of temperature. This assumption fits well for the reactions of small molecules in aqueous solutions, but enzyme-catalyzed reactions involve interactions between the substrate and a complex macromolecular enzyme that can undergo large changes in heat capacity (C_P) during catalysis. The macromolecular rate theory uses the fundamental equations of statistical thermodynamics to show that the large ΔC_P associated with macromolecular reactions leads to temperature dependence of $\Delta G^{\ddagger}$ (LiCata and Liu, 2011).

If $\Delta G^{\ddagger}$ is temperature dependent and particularly if ΔC_P is large and negative, then it is easy to show mathematically that the shapes of the TPCs for enzymes should exhibit a clear T_{opt} followed by declines in enzyme activity with increasing temperature, without having to invoke any denaturation of the enzyme. Similarly when ΔC_P is large and negative, thermal sensitivity will be greater at low temperatures – a pattern that does not conform to the predictions of the Arrhenius equation, but is frequently observed for biochemical reactions, including those associated with aerobic metabolism (Hochachka and Guppy, 1987). It is likely that ΔC_P varies among enzymes, which would result in differences in the shapes of the TPCs for different enzymes. Thus this theory provides a mechanistic basis for the TPC shape and its variation among proteins.

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Although they posit different mechanisms to account for the effects of temperature on protein activity, all of these models focus on the effects of temperature on maximal activity (V_{max} or k_{cat}). At V_{max} , saturating levels of substrate are provided and reaction rate is primarily limited by the rate of conversion of substrate to product rather than by substrate binding. But this does not capture biological reality, because substrate concentrations *in vivo* are low. This observation serves to highlight the importance of another critical parameter from classical enzyme kinetics – the Michaelis constant (k_m), which is an indicator of the interaction between the enzyme and its substrate. Michaelis constants are also dependent on temperature, with increasing temperature generally causing an increase in the apparent k_m (Somero, 1995; Somero et al., 1996; Hochachka and Somero, 2002). This effect of temperature on the k_m is thought to be due to alterations in protein conformation (i.e. shifts between protein microstates) that affect the ability of the protein to bind to substrate and catalyze conversion to product.

In general, organisms from warmer habitats tend to have enzymes that function at higher temperatures than do organisms from colder habitats, and this adaptation to high temperature has come at a cost such that these enzymes do not function as well at low temperatures (Somero, 1995; Wintrode and Arnold, 2001; Fields, 2001). From a mechanistic perspective, this pattern has usually been interpreted as a trade-off between flexibility and stability, such that the high structural stability that is needed to withstand high temperatures results in reduced flexibility that limits catalytic capacity at lower temperatures (Fields, 2001). Interestingly, however, it is possible to use *in vitro* directed evolution to 'design' enzymes with both excellent high-temperature stability and high catalytic activity at lower temperatures (Wintrode and Arnold, 2001; Arnold et al., 2001; Dean and Thornton, 2007; Kaltenbach and Tokuriki, 2014), which suggests that the stability–flexibility trade-off is not a biophysical constraint. But why, then, is the apparent trade-off between these properties so ubiquitous in nature?

There are at least two possible explanations for this observation. First, the lack of natural enzymes with these properties could be the result of neutral processes. There are very few possible structures that allow both stability at high temperatures and good catalytic activity at low temperatures, and so most mutations will tend to yield enzymes that lack these dual properties (Wintrode and Arnold, 2001; Harms and Thornton, 2013). In the absence of strong selection for both properties, random mutation will tend to remove these enzymes from the population. Alternatively, there may be functional trade-offs associated with maintaining both stability and flexibility that are not fully captured in the laboratory evolution experiments. For example, the lab-evolved proteins that are stable at high temperatures and active at low temperatures often have very low k_m compared with the naturally evolved proteins (Wintrode and Arnold, 2001; Arnold et al., 2001). It is possible that these changes in substrate binding properties are deleterious in natural environments, thus accounting for the lack of naturally evolved proteins with both high thermal stability and high catalytic activity at low temperatures.

There is also significant variation among proteins in the upwards slope of the TPC, but whether this variation has a consistent relationship with habitat is less clear. A recent meta-analysis of the upwards slope of TPCs for enzymes from bacteria adapted to cold, moderate and high temperatures failed to find any consistent relationship between these slopes and habitat temperature (Elias et al., 2014), although studies in other organisms have done so (Dahlhoff et al., 1991; Feller and Gerday, 2003). Similarly, the empirical data comparing stenotherms and eurytherms have sometimes (e.g. Hardewig et al., 1999), but not always (Swimmer et al., 2004) detected a reduced slope in enzymes from eurythermal organisms. Thus, it is difficult to conclude that selection acts strongly to modify the upwards slope of TPCs for proteins.

Decades of elegant studies using both intra-specific (e.g. Place and Powers, 1979) and inter-specific comparisons (e.g. Dahlhoff and Somero, 1993; Somero et al., 1996) provide convincing evidence that k_m demonstrates thermal adaptation. For example, multiple studies have demonstrated that the k_m values of enzymes from eurythermal organisms tend to be less thermally dependent than those from stenothermal organisms. Similarly, there is a general pattern of conservation of k_m such that the k_m values of homologous enzymes of organisms from different habitats are similar when assessed at the relevant operating temperature for the species (Somero, 1995; Fields, 2001). Thus, any comprehensive consideration of the effects of temperature on biological processes must consider, not just the effects of temperature on enzyme activity, but also the effects of temperature on enzyme–substrate interactions. From the perspective of the macromolecular rate theory, this could involve including a term capturing the effects of the large ΔC_P that occur during substrate binding, which has yet to be incorporated into the theory.

The discussion above has focused primarily on enzymes that operate in the aqueous environments of the cell, but many of the essential enzymes involved in aerobic metabolism are embedded in the mitochondrial membrane. Membrane properties such as fluidity are strongly affected by temperature (Cossins, 1983; Hazel, 1995) and these changes in membrane fluidity can have direct effects on the activity of membrane-embedded proteins (Somero, 2011). Indeed, at least in some cases, disruption of lipid–protein interactions has been suggested to be the cause of high-temperature-induced failure of mitochondrial respiration (e.g. O'Brien et al., 1991). The interactions between proteins and their membrane environments can result in complex shapes for the TPCs of these proteins and may influence properties such as k_m. This observation serves to point out the importance of the local environment around a protein, and is a cautionary tale for both membrane-embedded proteins and for cytosolic proteins alike. It also points out a more general caveat associated with measurements of TPCs on isolated enzymes, because activities measured *in vitro* often do not match those measured *in vivo* (Wright et al., 1992; Teusink et al., 2000).

Effects of temperature at the cellular and subcellular levels

It is clear that there is still a great deal to be learned about the factors that shape TPCs, even for individual proteins, particularly those involved in aerobic metabolism, and mechanistic understanding is even less well developed at higher levels of biological organization such as complex biochemical networks and intact cells. There have been some measurements of the effects of temperature on aerobic metabolism at the cellular level in animals (Somero and DeVries, 1967; Hoskins and Aleksiuk, 1973; Jorjani and Ozturk, 1999) and some data sets conform well to the Arrhenius equation and others do not. Similar patterns occur for measurements of oxygen consumption by isolated mitochondria (van den Thillart and Modderkolk, 1978; Somero et al., 1996; Weinstein and Somero, 1998; Hardewig et al., 1999; Abele et al., 2002; Somero, 2002; Johnston et al., 1994; Fangue et al., 2009; Hilton et al., 2010; Iftikar et al., 2010, 2014; Iftikar and Hickey, 2013). For example, Fig. 2 shows the TPC for oxygen consumption of isolated mitochondria from *F. heteroclitus*, assayed under conditions of saturating substrate. In this case, the data conform well to the predictions of the Arrhenius equation, but acclimation to low temperatures can result in substantial discontinuities in the TPC (Fangue et al., 2009).

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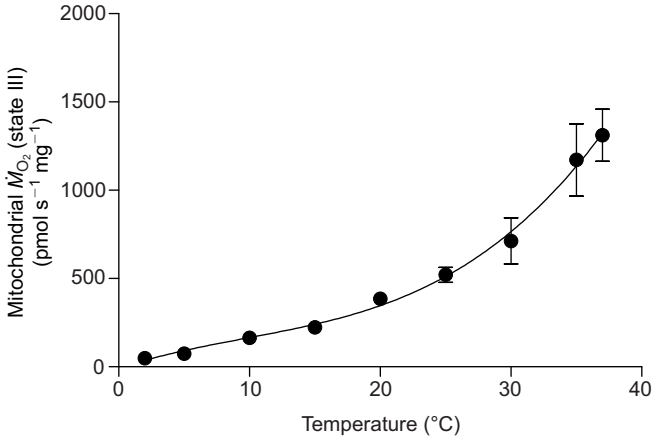

Fig. 2. A TPC for the rate of oxygen consumption of mitochondria from the eurythermal killifish *Fundulus heteroclitus*. Maximum ADP-phosphorylating rates of oxygen consumption (state III) with pyruvate as a substrate. Mitochondria were isolated from the livers of fish acclimated to 25°C (data from Fangue et al., 2009). Values are means±s.d.

Data that conform to the Arrhenius equation are often explained by assuming that the shape of the TPC for aerobic metabolism at the cellular level is governed by the thermal dependence of a single underlying process, for example, a rate-limiting step in the biochemical network (Corkrey et al., 2012). Similarly, cases in which significant discontinuities (ABTs) below the T_{opt} are detected in the TPCs are often attributed to the effects of temperature-induced changes in membrane structure that influence the function of the mitochondrial electron transport chain (O'Brien et al., 1991) and thus these explanations also tend to focus on single key processes and their interaction with the cellular environment. However, it is unclear whether assuming that a single key process determines the shape of the TPC for aerobic metabolism at the cellular level is justified.

Aerobic metabolism is a multistep process involving elaborate biochemical networks in both the cytoplasm and mitochondria. The control of flux through such biochemical networks is likely to be determined at multiple steps, rather than by a single 'rate-limiting' process (Kacser and Burns, 1973; Fell, 1997; Bruggeman and Westerhoff, 2007). Why then would aerobic metabolism as a whole ever show Arrhenius-like behavior in response to increasing temperature, rather than always exhibiting discontinuities? It is possible that that the various flux-controlling steps all have similar thermal dependencies in a given organism (Chaui-Berlinck et al., 2004). In support of this idea, analysis of anaerobic glycolysis in yeast (*Saccharomyces cerevisiae*) suggests that the activities of all the glycolytic enzymes in this species have similar thermal dependencies (Cruz et al., 2012). However, other studies have obtained different results (Postmus et al., 2012). Even less information is available for the enzymes involved in aerobic metabolism, but at least some studies have observed differences in the slope of the TPC for different mitochondrial enzymes (Lenaz et al., 1972; Wodtke, 1976; Irving and Watson, 1976). Even if the individual enzymes within a biochemical network have different thermal dependencies, it could still be possible to observe a relatively smooth TPC for the process as a whole, if a large number of steps share control of the overall process and there are no major temperature gaps between optima of the underlying curves. At the moment, we simply do not have sufficient data on the characteristics of biochemical networks to distinguish between these possibilities.

The analyses discussed above have attempted to model the systems properties of biological networks using data on the activity of proteins measured *in vitro*. *In vivo*, flux through metabolic pathways is most often controlled by variation in the concentrations of substrates, products or allosteric effectors, in what has been termed 'metabolic regulation' (ter Kuile and Westerhoff, 2001; Suarez and Moyes, 2012). Indeed, studies of the thermal dependence of glycolytic flux in yeast suggest that metabolic regulation is the dominant force in determining the response of pathway flux as temperature increases (Postmus et al., 2008). Consideration of this perspective on metabolic control suggests that it is not possible to develop a comprehensive mechanistic understanding of how aerobic metabolism responds to temperature simply by considering the effects of temperature on individual proteins without considering the role of substrates, products and allosteric effectors, and how they are affected by temperature. Understanding this process will require additional empirical studies of the effects of temperature on pathway flux, enzyme activities and metabolites at the cellular level.

Technological advances are now enabling the use of high-throughput methods that allow multiple biological processes to be assessed simultaneously. For example, this approach has been applied to the effects of temperature on growth in *Escherichia coli* (Chang et al., 2013), identifying cofactor synthesis as the most limiting process at high temperature. Such 'systems biology' approaches, coupled with analytical methods such as hierarchical analysis (ter Kuile and Westerhof, 2001), hold the promise of disentangling the mechanisms via which temperature affects complex metabolic networks and intact cells (Bordbar et al., 2014). However, remarkably few studies have examined the effects of temperature at this level of biological organization, and this area represents a fruitful avenue for future research, both into the acute effects of temperature on aerobic metabolism and on the responses that cells use to cope with these effects.

Effects of temperature at the organismal level

TPCs for aerobic metabolism at the organismal level have been characterized for many species (e.g. Clarke and Johnston, 1999; Sokolova and Pörtner, 2003; Giomi and Pörtner, 2013). Fig. 3

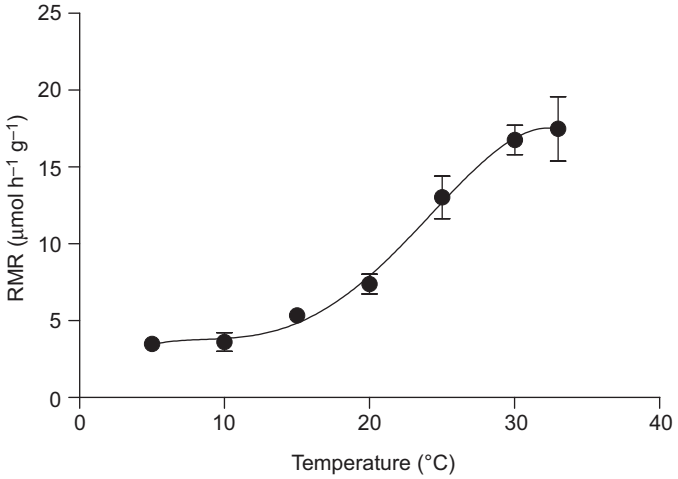

Fig. 3. A TPC for whole-animal routine aerobic metabolism in *Fundulus heteroclitus*. Routine metabolic rate (RMR) is the rate of oxygen consumption when the fish is resting quietly in the respirometer, but not necessarily at a minimum level of activity (data from Healy and Schulte, 2012). Values are means±s.e.m.

shows an example for the routine metabolic rate (RMR) of a highly eurythermal killifish, *Fundulus heteroclitus* (Healy and Schulte, 2012). This TPC conforms to the general shape of the TPCs shown in Fig. 1 but in this case, the falling phase of the curve could be not be measured because the fish reach their critical thermal maximum (CT_{max}) at temperatures only slightly higher than those shown here (Fangue et al., 2006). Based on the observation that prokaryotes and unicellular eukaryotes can tolerate significantly higher temperatures than those tolerated by metazoans, it has been argued that levels of organization above that of a single cell must be critical in determining the upper thermal limits of intact animals (Pörtner, 2002a; Storch et al., 2014). Among the mechanisms that have been suggested are the failure of system-level neural processes such as the regulation of ventilation and circulation by the brain (Lagerspetz, 1974), failure of cellular-level neural processes such as action potential conduction or synaptic transmission (Gladwell et al., 1976; Cossins et al., 1977; Rosenthal and Bezanilla, 2000; Robertson, 2004; Miller and Stillman, 2012), failure to maintain muscle membrane potential (Hosler et al., 2000), failure of membrane-associated processes more generally (Hulbert, 2003), failure of cardiac function (Pörtner and Farrell, 2008; Eliason et al., 2011) and failure at the level of the mitochondria (Pörtner et al., 2000; Pörtner, 2002a,b; Iftikar and Hickey, 2013).

However, the complex interdependencies of aerobic metabolism make it challenging to pinpoint the precise level of biological organization at which failure occurs and suggest that combinations of factors are likely to be involved. For example, cardiac or neural failure at the biochemical or cellular level could result in a failure of oxygen supply to the tissues, which would constrain aerobic metabolic rate at the whole-organism level. Similarly, failure of key controlling processes in the brain could cause reductions in cardiac or ventilatory capacity, constraining oxygen supply, and causing mitochondrial failure and the collapse of aerobic metabolism. Thus, aerobic metabolism could fail in response to acute high temperatures because of the failure of any of the processes involved in establishing either oxygen supply or demand at multiple levels of organization, and the positive feedback cycle between these processes could lead to rapid and catastrophic system failure with acute exposure to high temperatures.

The second important feature of TPCs for aerobic metabolism at the organismal level is the shape of the curve as oxygen consumption rises up to the T_{opt} (Fig. 3). The slope of this rising phase varies among species (Dell et al., 2011) and possibly among traits (Amarasekare and Savage, 2012), but whether this variation occurs in a consistent way between organisms that are adapted to different temperatures has been a matter of debate for more than 50 years (Scholander et al., 1953; Rao and Bullock, 1954; Clarke and Johnston, 1999). Testing the Arrhenius prediction of an exponential rising phase is essential, because theories such as the MTE assume that metabolism can be modeled based on the Arrhenius equation. Unfortunately, testing this prediction is far from straightforward as it is not clear whether measurements of metabolic rate at the organismal level actually have sufficient resolution to distinguish among alternative models for the shape of this slope. For example, using an extensive dataset on the metabolic rates of teleost fishes, Clarke and Johnston (1999) showed that the relationship between resting metabolic rate and temperature could be described equally well by several different curve fits. Similarly, some studies have demonstrated patterns in the thermal dependence of aerobic metabolism that can be adequately described by the Arrhenius equation (Aleksiuk, 1971; Stamou et al., 1995; Lee et al., 2003; Clarke and Johnston, 1999; Dahlhoff and Somero, 1993) while others have not (Giomi and Pörtner, 2013; for some classic examples in fish see Fry and Hart, 1948; Beamish, 1964). Overall, the empirical data suggest that TPCs for aerobic metabolism at the organismal level can, but do not always, conform to the predictions of the Arrhenius equation, and that there may be differences in these shapes between standard and maximum metabolic rate (Pörtner, 2002a,b). At the organismal level there are complex homeostatic mechanisms involving the nervous and endocrine systems that can act to shape the response of metabolic rate to increasing temperatures, which have the potential to alter the shape of the TPC across various time scales (Schulte et al., 2011). Thus, the available data do not conform well to the assumptions of models such as the MTE. This observation raises the more general question of whether we would actually expect a complex, organismal-level process such as aerobic metabolism to conform to the predictions of a theory that was generated to explain the behavior of individual chemical reactions. In fact, extending a theory developed for individual reactions even to the level of cells or biochemical networks can be problematic.

Metabolism, aerobic scope and climate change

Although theories such as the MTE and OCLTT use the effects of temperature on aerobic metabolism as a fundamental building block of their predictions about the impacts of climate change, this only provides a partial picture of the effects of temperature on metabolism. Oxygen consumption is a good index of flux through the mitochondrial electron transport chain, but is not necessarily a good indicator of ATP production across temperatures because of the effects of temperature on proton leak across the mitochondrial inner membrane. Proton leak allows protons to move across the mitochondrial inner membrane via pathways other than the ATP synthase, thus dissipating the proton motive force and driving oxygen consumption without generating ATP. Substantial amounts of data suggest that the thermal sensitivities of ATP generation and proton leak differ (Hardewig et al., 1999; Hilton et al., 2010; Iftikar et al., 2010, 2014; Iftikar and Hickey, 2013) because of increases in proton permeability due to changes in membrane fluidity at higher temperatures (Seebacher et al., 2010; Zukiene et al., 2010). As a result, proton leak represents an increasing fraction of oxygen consumption as temperature rises (Hilton et al., 2010; Seebacher et al., 2010; Iftikar and Hickey, 2013) causing reductions in the efficiency of mitochondrial ATP production with increasing temperature. These effects are considered within the OCLTT but are largely neglected by the MTE.

It is also important to consider the potentially deleterious side effects of mitochondrial respiration. Flux through the electron transport chain results in the formation of reactive oxygen species (ROS) that have the potential to damage cellular macromolecules such as lipids, proteins and DNA (Murphy, 2009; Tomanek, 2015). ROS also act as potent signaling molecules within the cell (Finkel, 2011) and thus may play a role in the regulation of diverse cellular processes. ROS production by the mitochondria increases with temperature (Abele et al., 2002), which could have an impact on the responses of organisms to temperature change (Heise et al., 2006; Blier et al., 2013).

Focusing on aerobic metabolism from the perspective of oxygen consumption also tends to emphasize the importance of energy supply, and potentially underestimates the contribution of pathways involved in energy demand (Hofmeyr and Cornish-Bowden, 2000). Metabolic control analysis suggests that the majority of control over flux through ATP-supply pathways resides in the demand of the cell for ATP (Koebmann et al., 2002), and ATP demand processes are temperature dependent. If demand processes

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are more thermally sensitive than the processes involved in energy supply, then changes in the activity of these demand processes will shape the TPC for aerobic metabolism. Thus, developing a full understanding of the responses of organisms to climate change requires an understanding of the effects of temperature on both energy supply and energy demand.

The OCLTT concept explicitly addresses this issue of supply and demand matching, advancing the hypothesis that it is ultimately a mismatch between demand for oxygen by the tissues and the capacity to supply oxygen by the cardiovascular system that limits function at high temperatures (Pörtner and Knust, 2007). The OCLTT suggests that this mismatch between supply and demand is most clearly indicated by a loss of aerobic scope at high temperatures and that this decline in aerobic scope is directly and causally related to declines in performance traits such as growth and reproductive success. This linkage has been convincingly demonstrated for a number of fish and marine invertebrate species (Pörtner, 2001, 2010; Eliason et al., 2011). However, there is limited support for aerobic scope limiting maximum thermal tolerance in air-breathing ectotherms (Fobian et al., 2014) and particularly in taxa such as insects (Klok et al., 2004; Stevens et al., 2010). In addition, recent work in a variety of species of fishes has indicated that, at least in some species, aerobic scope can still be high at temperatures at which growth and reproduction are compromised, suggesting that thermal limitations on aerobic capacity cannot be the direct cause of fitness limitations in these species (Healy and Schulte, 2012; Clark et al., 2013a; Gräns et al., 2014; Norin et al., 2014). As a result of these divergent observations, there is currently a very lively debate in the literature with respect to the relevance of the OCLTT for predicting the responses of species to climate change (Clark et al., 2013a,b; Pörtner and Giomi, 2013; Farrell, 2013).

Many of the data sets to which the OCLTT has been successfully applied have TPCs for standard metabolic rate (SMR) and maximum metabolic rate (MMR) of the shape shown in Fig. 4A. In these TPCs, MMR reaches a limit at a lower temperature than does SMR, causing aerobic scope to decline at a relatively low temperature (Fig. 4B). However, other patterns are possible. Fig. 4C shows the general shape of the TPCs for SMR and MMR based on data for *F. heteroclitus* (Healy and Schulte, 2012). In this case, both SMR and MMR increase exponentially, essentially in parallel, up to temperatures close to the maximum temperature that can be tolerated (even acutely) by the organism, resulting in a broad aerobic scope curve (Fig. 4D). The comparison between the two types of aerobic scope curves shown in Fig. 4 serves as a reminder that aerobic scope is a composite trait resulting from the effects of temperature on SMR and MMR, and these rates are likely to be under different constraints and respond independently to selection.

In species with aerobic scope curves similar to those shown in Fig. 4D, there is little evidence of a limitation by oxygen supply as temperature increases, nor is there a clear link between the position of the aerobic scope curve and the temperatures that maximize fitness-related traits such as growth (Healy and Schulte, 2012; Donelson et al., 2014; Khan et al., 2014; Norin et al., 2014; Gräns et al., 2014). Instead, growth and development have thermal optima well below the upper end of the aerobic scope T_{opt} window in these species. For example, our work on *F. heteroclitus* indicates that at temperatures where both RMR and MMR are still increasing exponentially with temperature, and aerobic scope is maximal, the fish have difficulty maintaining body mass during long-term acclimation (Healy and Schulte, 2012). These data point to limitations on the ability to take up, process or assimilate sufficient nutrients to support the high metabolic rates typical of high temperatures. Similar mismatches between the rates of nutrient acquisition and the rate of metabolism with increasing temperature have been observed in a variety of species (Lemoine and Burkepile, 2012; Rall et al., 2010; Alcaraz et al., 2014), suggesting a supply and demand mismatch that is not necessarily reflected in a loss of overall aerobic scope. This means that making a direct connection between an aerobic scope curve and fitness may be difficult in these organisms.

A similar disconnect occurs when attempting to link the patterns obtained from an aerobic scope curve and other life history traits. For example, the thermal window for reproduction is often much narrower than the thermal window for activity (Fry, 1971) and thus the temperature at which the maximum aerobic scope attainable during exercise starts to decline cannot always be used as an index of the temperatures that compromise fitness. Similarly, failure of complex cellular-level processes, such as the cell cycle, occurs at moderate temperatures (van der Have, 2002), which could be a

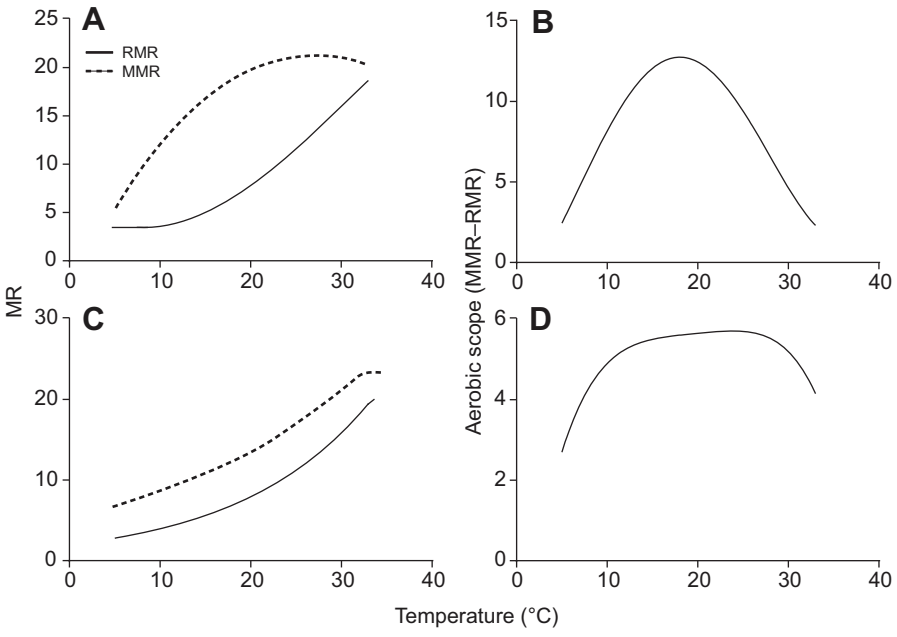

Fig. 4. Two main types of aerobic scope curve in fishes. (A) RMR (routine metabolic rate) and MMR (maximum metabolic rate), where MMR is most likely limited by oxygen supply at moderate temperatures. (B) Aerobic scope curve (MMR–RMR) for the data in A. (C) RMR and MMR where there is no evidence that MMR is limited by oxygen supply except at thermal extremes. (D) Aerobic scope curve (MMR–RMR) for the data in C.

reason why the tolerance windows for development are narrower than adult tolerance windows. Ultimately, the shape of the TPC for aerobic metabolism at the organismal level is likely to be influenced by both supply and demand processes acting across multiple levels of organization and must be the product of a range of energy allocation 'decisions' made by the organism under the complex combinations of biotic and abiotic factors that characterize natural environments. Many of these issues are considered within the OCLTT, but the connection between these energy allocation decisions and aerobic scope has not always been made clear within the theory or has been misinterpreted by those attempting to apply it. Thus, the primary debate surrounding the OCLTT is not focused on the mechanistic underpinnings of the theory, but rather on the suggestion that aerobic scope is an adequate predictor of these challenges. In fact, different conceptions of what is meant by the term aerobic scope may underlie some of the debate about the concept (Clark et al., 2013a,b; Pörtner and Giomi, 2013; Farrell, 2013). The variety of patterns illustrated in Fig. 4 indicate that taking a broad view of metabolism that encompasses the many factors that can influence energy supply and demand across different time scales (e.g. Sokolova et al., 2012; Sokolova, 2013) is the most appropriate way in which to view the relationship between aerobic metabolism and temperature at the organismal level.

Conclusions and perspectives

Although studies of biochemical adaptation have successfully demonstrated many of the mechanisms that organisms use to cope with the effects of temperature on biological processes, we still lack a comprehensive mechanistic understanding of the effects of temperature on biological functions, even at the level of single proteins, and it remains unclear how these effects on proteins and biological membranes are integrated to affect processes across levels of organization. The ways in which temperature affects complex biochemical networks such as aerobic metabolism at the cellular level are particularly unclear. One of the most challenging aspects of thinking about the effects of temperature on metabolism is distinguishing cause from effect and disentangling the relationship between supply and demand processes (Glazier, 2015) across multiple time scales (Schulte et al., 2011). Developing a fuller understanding of these issues is an important challenge for thermal biologists and will be essential if we are ever to develop a truly mechanistic understanding of the responses of species to climate change.

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Competing interests

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