Functional Ecology 2006

FORUM

Response to Clarke and Fraser: effects of temperature on metabolic rate

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Introduction

In two recent papers, Clarke and Fraser (2004) and Clarke (2004) discussed the empirical data and the mechanistic processes relating metabolic rate to temperature. They criticized the framework proposed recently by Gillooly *et al.* (2001) and presented an alternative evolutionary trade-off hypothesis.

Gillooly *et al.* (2001) (see also West, Brown and Enquist 1997; Gillooly *et al.* 2002; Charnov and Gillooly 2003; Brown *et al.* 2004a,b) developed a theory for the scaling of metabolic rate that *combines* the effects of two primary variables, body size and temperature, based on first principles of physics, chemistry and biology – including the fitness-maximizing dynamic of natural selection. This leads to a single equation for whole-organism metabolic rate, B:

$$B = b_0 M^{3/4} e^{-E/kT}, \qquad \text{eqn 1}$$

where b_0 is a normalization constant, and M is body mass. The Boltzmann–Arrhenius factor, $e^{-E/kT}$, characterizes the exponential effects of temperature, where E is the activation energy, k is Boltzmann's constant ($8{\cdot}62 \times 10^{-5}$ eV K^{-1}) and T is absolute temperature in degrees kelvin.

While this theory builds on longstanding physiological research showing that metabolic rate scales as a power function with body mass and exponentially with temperature, it differs from previous treatments in the following three ways. First, it *derives* the 3/4 exponent of the body mass term, based on the fractal-like design of biological resource distribution networks (West *et al.* 1997). Second, it *predicts* that E takes on a limited range of values, 0·6–0·7 eV with an average of about 0·65 eV, reflecting an average activation energy of the biochemical reactions of respiration (Gillooly *et al.* 2001). The Boltzmann–Arrhenius factor in the second term of equation 1 incorporates both the general theory for the kinetics of chemical reactions (Boltzmann 1872), and the empirically determined activation energies for the critical reactions of respiration, which have been known since at least Crozier (1924). Third, equation 1 combines the effects of size and temperature in a single, simple analytical expression that has just one free parameter, b_0, and two derived parameters, 3/4 and ~0·65 eV. This expression makes quantitative, *a priori* predictions that are strongly supported by empirical data (Fig. 1).

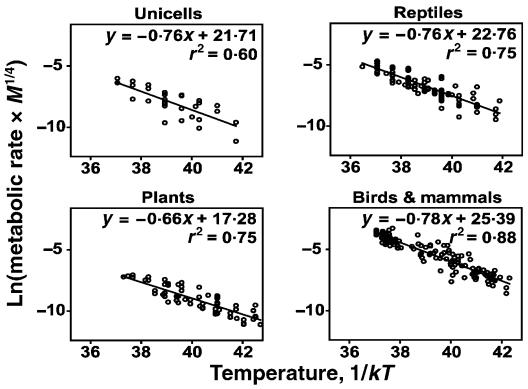

Fig. 1. Plot of the logarithm of mass-corrected basal metabolic rate *vs* temperature ($1/kT$), where k is Boltzmann's constant and T is absolute temperature in Kelvin (equation 1), for unicells, plants, reptiles, and birds and mammals. Data for birds and mammals include normal body temperatures and lower temperatures during hibernation and torpor. Data from Gillooly *et al.* (2001).

Points of contention

Clarke and Fraser take exception to the universal temperature dependence embodied in the second term of equation 1. While they recognize that Gillooly *et al.* (2001) build directly on the well-established Boltzmann–Arrhenius principle that biochemical reaction rates increase exponentially with temperature (Boltzmann 1872; van't-Hoff 1884; Arrhenius 1915), they criticize our use of this first principle of physical chemistry. Here we respond to the issues that they raise.

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(1) WHY USE THE BOLTZMANN RELATION?

Clarke and Fraser (2004) argue that our model, which uses the Boltzmann–Arrhenius relation, is simply a statistical description of the temperature dependence of metabolic rate, and therefore not preferable to other expressions such as the Q_{10}, or even the logarithm of rate as a function of the logarithm of temperature. This represents a change of viewpoint for Clarke, who in 1999 concluded that 'in the absence of any alternative theory or explanation, the Arrhenius relationship is therefore probably the most appropriate statistical description of whole-animal thermal physiology' because 'it is based firmly on statistical thermodynamics' (Clarke and Johnston 1999, p. 900; see also Clarke 1993). Indeed, Gillooly *et al*. (2001) use the Boltzmann–Arrhenius expression because of its foundation in statistical thermodynamics. This form also avoids inherent errors associated with Q_{10}. As temperature varies but the activation energy remains constant, Q_{10} deviates systematically from the exact expression: for $E = 0{\cdot}65$ eV, the Q_{10} approximation decreases by 20%, from 2·75 to 2·16, over the biological temperature range of 0–40 C ($T = 273$–313 K; $Q_{10}(T) \approx e^{10E/kT^{\wedge}2}$

Therefore, not only is the Boltzmann relation more firmly based in statistical thermodynamics than the Q_{10} and the other alternative functions suggested by Clarke, it is also more accurate than the Q_{10}.

(2) DO OTHER PHENOMENA OVERRIDE THE EFFECT OF SIMPLE BOLTZMANN KINETICS?

Clarke and Clarke and Fraser claim that equation 1 and the simple Boltzmann factor do not apply to whole-organism metabolic rate, because the complexities of intermediary metabolism override the direct effects of temperature on biochemical reactions. To support their claim, they expound at length on the many processes and variables that are relevant to effects of temperature on metabolic rate. These include proton leak, relative effects of enthalpy and entropy, changes in protein structure and function, and various environmental factors, including variation in food supply. However, just pointing out that these factors can 'play a role', does not constitute evidence that they are of sufficient quantitative magnitude to override Boltzmann kinetics. If these other factors are of primary importance, then why do Arrhenius plots, which are based explicitly on the Boltzmann factor with a predicted value for E, provide such excellent fits to the data? Our plots of multiple data sets (e.g. Figure 1 and others in Gillooly *et al*. 2001) show that, after correcting for body size, a linear regression with slope $-E$ typically accounts for 60–90% of the variation in the temperature dependence of metabolic rate across species. The similarity of these plots for numerous taxa adapted to diverse thermal environments shows that there is a very general temperature dependence, with values of E usually in the range 0·6–0·7 eV, despite the unique species-specific influences of 'temperature, ecology and life history' emphasized by Clarke and Fraser.

(3) WHAT IS THE MECHANISM UNDERLYING OUR USE OF THE BOLTZMANN RELATION?

Clarke cites the above complexities to argue that our formulation of temperature dependence of metabolic rate is not mechanistic. Although many of the processes linking whole-organism metabolism to the underlying biochemistry remain to be elucidated, important linkages have been identified. Our model assumes that the exponential effect of temperature on whole-organism metabolic rate reflects the underlying statistical thermodynamics of the chemical reactions of the TCA cycle that occur in the mitochondria. About 80% of the oxygen consumed by an organism is used by the mitochondria to produce ATP (Hochachka and Somero 2002).

The relatively limited available data on the temperature dependence of mitochondrial respiration and ATP synthesis *in vitro* show a similar temperature dependence to whole-organism metabolic rate. Guderley (2004) found that rates of substrate oxidation in isolated mitochondria for different fish species from very different thermal environments are described by a *single* temperature-dependent relationship ($E \approx 0{\cdot}5$ eV). This is very similar to that reported by Gillooly *et al*. (2001) for whole-organism metabolic rate in fish ($E \approx 0{\cdot}5$ eV), although it is somewhat lower than the predicted 0·6–0·7 eV. Blier and Guderley (1993) measured rates of ATP production in isolated mitochondria in rainbow trout at three temperatures (8, 15 and 22 C) and a broad range of substrate (ADP) concentrations, much broader than probably holds *in vivo*. We reanalysed their data for the six non-saturating ADP concentrations (40–500 nmol l^{-1}), assuming that ADP is non-saturating in natural conditions. A plot of these data *vs* $1/kT$ yields an activation energy, $E = 0{\cdot}66$ eV (Fig. 2, $r^2 = 0{\cdot}55$). Note that the different ADP concentrations had a modest effect on the activation energy (i.e. slopes ranged from 0·52 to 0·80), but a pronounced effect on the normalization constant (i.e. the intercept). Under all 10 ADP concentrations (40–2000 nmol l^{-1}) used by Blier and Guderley (1993), which includes both saturating and non-saturating conditions, the average activation energy was 0·56 ($r^2 = 0{\cdot}40$), although the range of slopes was more variable (0·38–0·8). These examples provide a counter to Clarke's claim that 'the simple link between resting metabolic rate and temperature proposed by Gillooly *et al*. (2001) is incompatible with what we know about the physical chemistry of enzyme catalysis.'

(4) IS METABOLISM CONTROLLED BY SUPPLY OR DEMAND?

Clarke and Clarke and Fraser suggest that ATP production is driven largely by the demand for energy to

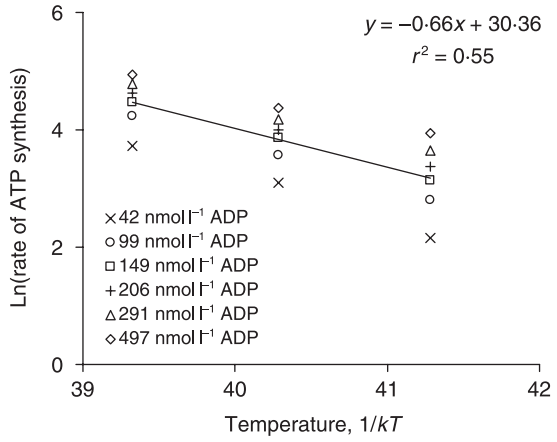

Fig. 2. Plot of the logarithm of rate of ATP synthesis (nmol min^{-1} mg^{-1} mitochondrial protein) *vs* temperature ($1/kT$) for isolated trout muscle mitochondria, measured at three different temperatures, 8, 15 and 22 C, and six different non-saturating substrate concentrations of free ADP. This shows the systematic effect of substrate availability on increasing the normalization constant, b_0. The slope of the fitted regression line across all six substrate concentrations gives the average activation energy, 0·66 eV, which is intermediate in the range 0·6–0·7 eV predicted by equation 1 and Gillooly *et al*. (2001). Reanalysis of data from Fig. 1 in Blier and Guderley (1993).

perform biological work rather than directly by temperature and thus by resource supply. The idea that metabolic rate is *either* demand *or* supply driven is a false dichotomy. The anatomies and physiologies of organisms have evolved under natural selection to match supply to demand and vice versa. An animal operating at a higher temperature with a higher metabolic rate not only demands more resources, it also has a greater capacity to supply – to find, capture and assimilate – resources.

(5) WHAT ARE THE EFFECTS OF ACCLIMATION, ACCLIMATIZATION AND ADAPTATION?

Clarke and Clarke and Fraser argue that acclimation, acclimatization and adaptation must be included in any general model of metabolic rate. This is surprising, because previously Clarke and others have presented data that strongly refute the metabolic cold adaptation hypothesis (e.g. Clarke 1993; Clarke and Johnston 1999; Steffenson 2002). Still, Clarke and Frasier argue that our model 'provides no opportunity or mechanism for laboratory acclimation, seasonal acclimatization or evolutionary adaptation, other than by a change in *E*.' This is incorrect. In fact, equation 1 predicts that *E* will remain relatively constant in the range of 0·6–0·7 eV, and any effects of acclimation, acclimatization and adaptation will be reflected predominantly in shifts in the normalization constant, b_0. Additionally, however, our deliberately simple model was not intended to describe all of the variation. We were aware that the processes of acclimation, acclimatization and adaptation complicate the responses of ectotherms to variation in environmental temperature, but we considered these to be among 'the secondary factors required to explain the remaining variation within and between groups.' Our viewpoint is supported by a recent meta-analysis by Addo-Bediako, Chown and Gaston (2002) of studies of insect metabolism (446 spp., 63 families, 77 range of latitude) which concluded that evolutionary adaptation had only a small effect. Our reanalysis of these insect data (their Fig. 2) showed that this effect, while statistically significant and biologically relevant, accounts for <4% of the variation in metabolic rate.

Our model does indeed allow for acclimation, acclimatization and adaptation, not through a change in *E* as Clarke and Fraser incorrectly assume, but through a change in b_0, the normalization constant, in equation 1. We have pointed out previously that b_0 is expected to vary among taxa and depending on environmental conditions (Gillooly *et al*. 2003). This is consistent with empirical observations that the primary effect of these processes is to reset the overall rate of metabolism, thereby changing the normalization of the Boltzmann factor. For example, in a recent review of the subject, Guderley (2004) concluded that rates of substrate oxidation from isolated mitochondria in fish show virtually no effects of evolutionary adaptation, but they do often show facultative compensation through mechanisms, such as increasing mitochondrial density, that shift the overall rate of metabolism – i.e. that change b_0. This is also clear in Fig. 2, which shows that changes in substrate concentration shift the vertical position of the Arrhenius plots, but cause only modest changes in *E* (slope).

(6) ARE EFFECTS OF TEMPERATURE THE SAME WITHIN AND ACROSS SPECIES?

Clarke and Frasier contend that our model requires that the temperature dependence of metabolic rate must be identical within species and across species. This is incorrect, in part because they ignore our admonition that there is indeed substantial variation in the relationships we have plotted. This variation is probably due to some combination of: (i) systematic displacement from the regression line due to differences in b_0, which may reflect effects of acclimation, acclimatization and adaptation (see above); and (ii) less structured deviations around the regression line due to other factors. It is straightforward to make an Arrhenius plot such as Fig. 1, quantify the unexplained variation around the regression line, and use the residuals to address hypotheses about acclimation, acclimatization, adaptation and other factors. For example, the classical metabolic cold adaptation hypothesis would predict little or no change in *E* (slope) but shifts in b_0, resulting in either positive or negative residuals in response to colder or warmer conditions, respectively.

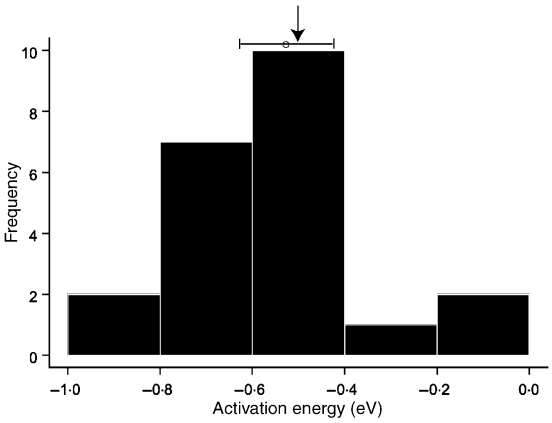

Fig. 3. A comparison of intra *vs* interspecific metabolic rates of fish as a function of temperature to test the evolutionary trade-off hypothesis of Clarke and Clarke and Fraser. The histogram shows frequency distribution of intraspecific slopes of Arrhenius plots for 23 species of fishes measured in the laboratory after exposure to different temperatures; the circle and horizontal line above the bars depict the mean and 95% CI, respectively. The arrow gives the interspecific slope for 68 species measured in the laboratory at the temperatures at which they live. These data do not support the prediction of Clarke's and Clarke and Fraser's evolutionary trade-off hypothesis that the intraspecific slope should be substantially greater than the interspecific slope. Data from Clarke (2004).

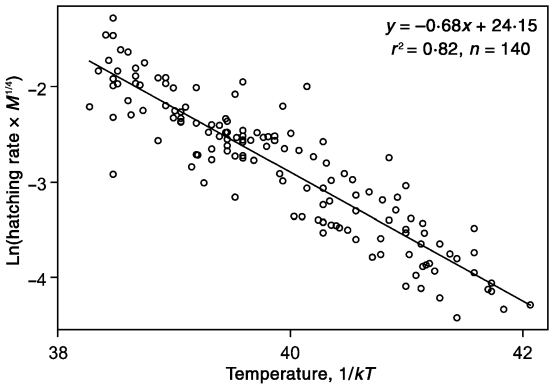

Fig. 4. Plot of the logarithm of mass-corrected hatching rate as a function of temperature ($1/kT$) for marine fish eggs in the field, where k is Boltzmann's constant and T is absolute temperature in kelvin. The slope is within the range –0·6 to –0·7 eV predicted from equation 1 from Gillooly *et al.* (2001).

In contrast to our model, the evolutionary trade-off hypothesis of Clarke and Clarke and Fraser is not clearly stated, makes no quantitative predictions, and is consequently difficult to test rigorously. Consequently, Clarke and Fraser create a plot of metabolic rate as a function of temperature with 'data points' for some hypothetical organisms. This graphical model predicts that a plot of metabolic rate *vs* temperature within species measured over short time intervals in the laboratory should be much steeper than a relationship across species with measurements made at the temperatures where the individuals normally live – and to which the species are presumably acclimated, acclimatized and adapted. To test this prediction and refute the 'tyranny of Boltzmann' embodied in our equation 1, Clarke (2004) presents the analysis of Clarke and Johnston (1999) that claims to compare the average intraspecific Q_{10} values obtained in 14 studies with the average interspecific Q_{10} value for '68 fish species' (which were held in the laboratory) 'at the temperatures at which they live'. This analysis is problematic for several reasons, including that the average intraspecific Q_{10} value is based on three intraspecific studies (not 14), and the majority of values come from just two species measured multiple times over the same temperature range. We reanalysed this same data set presented in Clarke and Fraser (2004) and Clarke (2004), but included all 24 species measured at two or more temperatures spanning at least 5 C, and excluded one outlier. We found that the average intraspecific slope of mass-corrected rates as a function of $1/kT$ was nearly identical to the interspecific slope (Fig. 3). So, Clarke and Fraser's own data do not refute the tyranny of Boltzmann, nor do they agree with their 'hypothetical data' or support their evolutionary trade-off hypothesis.

(7) IS BOLTZMANN TEMPERATURE DEPENDENCE MALADAPTIVE?

Clarke and Clarke and Fraser argue that direct Boltzmann temperature dependence is usually maladaptive, because it is wasteful of food resources. Although this is undoubtedly true in some cases, there may more often be fitness gains associated with operating at higher metabolic rates at higher temperatures. This is suggested by the very similar exponential temperature dependence that holds across many diverse kinds of organisms and over virtually the entire biological temperature range (0–40 C) – far greater than a typical species ever experiences over its geographical range or the lifetime of an individual. So, for example, the development rates of fish eggs reflect the rates at which the embryos convert stored egg resources into larval biomass, so they integrate metabolic rate over the developmental period. An Arrhenius plot following equation 1 shows that over almost a 30 C range in temperature, hatching rates for 140 species of marine fishes in the field exhibit very similar temperature dependence, with the regression giving an activation energy of 0·68 eV and accounting for 82% of the variation (Fig. 4; data from Pauly and Pullin 1988). So in many cases, Boltzmann may be a welcome tyrant.

Conclusions

Clarke (2004) and Clarke and Fraser (2004) criticize Gillooly *et al.* (2001) (and by implication, subsequent papers by our group on metabolic theory). They argue that it is neither theoretically justified nor biologically realistic to use the Boltzmann factor and our equation 1 to describe the effect of temperature on metabolic rate. We respond to all of their important criticisms. Despite recent important advances in

understanding the phenomena of thermal acclimation, acclimatization and adaptation at molecular to whole-organism levels, there is no reason to abandon a century of theoretical and empirical research demonstrating the fundamental importance of the Boltzmann expression. It characterizes the direct exponential effect of temperature on kinetics of biochemical reactions, organelle- and cellular-level processes, whole-organism metabolic rates and many other biological activities controlled by metabolic rate.

Acknowledgements

We thank W. Woodruff for comments, and A. Clarke for graciously providing us with his data. We all thank the National Science Foundation, and JFG, GBW and JHB are grateful for support from the Thaw Charitable Trust and a Packard Interdisciplinary Science Grant.

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Received 16 June 2005; revised 28 July 2005; accepted 12 August 2005
Editor: Steven L. Chown

doi: 10.1111/j.1365-2435.2006.01110.x