From movement to predation: metabolism rules all

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**Introduction**

The past 50 years revealed the fastest rise in temperatures ever recorded (Houghton, 1996; Stocker, 2014), raising questions on the effect these environmental changes are having on the wide range of ecological processes and biological traits directly dependent upon temperature (Coley *et al.*, 1985; Loreau *et al.*, 2001). Temperature is arguably the most important abiotic factor directing ecological processes, from individual metabolism (Gillooly *et al.*, 2001) to ecosystem dynamics (Saxe *et al.*, 2001; Yvon-Durocher *et al.*, 2012). This rapid increase in temperatures is having a direct impact on species and their interactions (Hughes, 2000; Penuelas and Filella, 2001). On a fundamental level, temperature affects biology via its effect on species’ metabolic rates (Gillooly *et al.*, 2001). Biochemically, as energy is provided by respiration, all biological traits depend on temperature and thus, species interactions, food webs and ecosystem dynamics are affected by the changes in species metabolic rates. Despite the body of work on how temperature affects metabolic rates (Brown *et al.*, 2004; Gillooly *et al.*, 2001), we still lack the necessary understanding to predict how future changes in global temperatures will affect species and their interactions at such a fundamental level.

Temperature performance curves (TPCs) of biological traits are key to understanding how temperature determines biological processes. The metabolic theory of ecology describes the relationship between temperature and metabolism (Brown *et al.*, 2004). This approach has been used both in studies of metabolic rate (Dell *et al.*, 2011; Gillooly *et al.*, 2001) and other biological traits (Savage *et al.*, 2004; Vucic-Pestic *et al.*, 2011). Metabolic rates show a unimodal relationship with temperature due to high temperature biochemical processes (Angilletta, 2006; DeLong *et al.*, 2017b; Figure 1a). Focusing on temperature performance curves of locally adapted populations has revealed various thermal adaptation scenarios (Kingsolver, 2009), which have higher-level effects on their population dynamics (Rall *et al.*, 2010; Vucic-Pestic *et al.*, 2011). Long-term changes in temperatures result in TPC adaptation patterns including displacement in temperature at peak performance (determined by *Tpk*, Figure 1b) and changes in the curve’s elevation (determined by the performance parameter *b0*, Figure 1c) (Kordas *et al.*, 2011). These adaptations have consequences on species interactions by producing short-term mismatches in performance between predators and their prey (Dell *et al.*, 2014). Most species currently perform differently throughout their operational temperature range (OTR) (Figure 1d), with prey usually under more pressure to perform at the bottom of the range (Dell *et al.*, 2011). As species adapt to new environments, new mismatches will arise and lead to new interaction patterns. These in turn will have an effect on community dynamics and could lead to changes in ecosystem assemblage and increased extinction risk (Albouy *et al.*, 2014; Rall *et al.*, 2010).

Comprehensive mechanistic modeling of the effect of metabolism on higher level processes promises to yield invaluable insight into the biochemical processes directing ecology (Dell *et al.*, 2014; Gibert and DeLong, 2014; Pawar *et al.*, 2015). Focusing on metabolic requirements of aquatic invertebrates, we develop a modeling framework to predict higher-level trait performance and species interactions. Such an approach provides a robust mechanistic alternative to time consuming complicated empirical work (Rall *et al.*, 2010). Bridging the gap between basal biochemistry and higher-level ecological processes will further our understanding of the effect of temperature and warming on biological systems. We support our theoretical approach by integrating empirical work to understand the relationship between biological traits and species interactions with temperature. We focus on three taxa of aquatic invertebrates found in the Iberian Peninsula. All individuals originate from a large mesocosm experiment set up in Spain and Portugal and covering a wide range of temperatures. All three species of interest are widespread insects in Europe and fill different ecological niches: top predator, intermediate consumer and bottom-feeder. These taxa have had time to acclimate to the thermally diverse region they inhabit enabling us to detect the longer-term acclimation effect of warming on their respective TPCs. We look for temperature-induced mismatches in the TPCs of a key biological trait, velocity, to predict changes in species interactions for locally adapted populations.

Velocity has been shown to be a key trait for directing predator-prey interactions (Dell *et al.*, 2014). As metabolism sets the pace for all biological rates (Brown *et al.*, 2004), velocity can be seen as a function of metabolic rate. In part, the energy produced from respiration will be used up for muscle contraction and locomotion. Research on animal locomotion suggests specific relationships between cost of transport and metabolic rate based on locomotion type (Alexander, 2003; Gibert *et al.*, 2016; Hein *et al.*, 2012; Tucker, 1970; Videler and Nolet, 1990; Videler, 1993). Thus, through temperature adaptation of this trait we expect to detect changes in relative performance of locally adapted populations, affecting their ability to forage or escape.   Predator-prey interactions are typically modeled using Holling’s type II functional response equation, dependent on key parameters: search rate and handling time (Holling, 1959). Search rates are determined both by the biological traits of the resource and its consumer and by environmental conditions. Biotic effects on search rates are determined by the relative velocity of the predator and its prey in random movement (Dell *et al.*, 2014). Both prey and predator velocities are biological rates that we expect to scale with mass and temperature in a similar way to metabolic rates (Brown *et al.*, 2004). Temperature dependence of search rates has been suggested (Rall *et al.*, 2012) but proper mechanistic understanding of the relationship is lacking. In addition to temperature, environmental dimensionality has been shown to have an effect on search rates (Pawar *et al.*, 2012). We thus combine our empirical metabolic approach to a new mechanistic model of search rates to make qualitative predictions on the effect of this temperature dependent change in performance on predator-prey dynamics.

To increase our understanding of the effect of temperature on multi-level ecological processes, we here investigate whether metabolic rates and their associated TPCs change between locally adapted populations, what parameters drive these changes and what effect these have on higher-level ecological traits and processes. We analyse respiration TPCs of locally adapted populations to (i) test for local changes in metabolic rates and (ii) look for their drivers. We then consider (iii) how changes in metabolic rates affect relative velocities of predator-prey pairs and (iv) how local adaptation to thermally diverse environments results in performance mismatches. Finally, we (v) consider the effects of metabolic rates adaptation to temperature on predator search rates over a temperature gradient.

**Materials and Methods**

STUDY SITES

A system of six sites spread throughout the Iberian Peninsula was used for this study (Figure 2). All sites included 32 artificial mesocosm ponds of 320L volume capacity set up and seeded in 2015 with an assemblage of locally sampled freshwater species. The communities in these ponds were left to assemble naturally and resulted in diverse macrophyte, and thus invertebrate, assemblages between ponds. Adult stages of most invertebrate species found in the ponds are efficient dispersers that can be found throughout ponds at each site (Matias, unpublished data). The sampling period for this study corresponded to the local spring season (April to May). The distinct geographical position of these sites exposed them to different local climates; two sites, Toledo and Murcia, were located in hot desertic regions (12°C to 20°C), two sites, Evora and Porto, in intermediate oceanic regions (10°C to 16°C) and two sites, Jaca and Penalara, in colder mountainous regions (6°C to 11°C). Mesocosm temperatures were recorded with submerged loggers every hour for the duration of the fieldwork at each location (4-6 days).

STUDY ORGANISMS

Experiments in this study were carried out on macro invertebrates native to ponds of the Iberian Peninsula. Species were chosen based on abundance and trophic level. Feeding trials were carried out at each site to assess species interaction. Potential predator species were left overnight in an arena with expected prey species. These trials revealed a predatory relationship between the dragonfly species *Sympetrum striolatum* and two prey items, the mayfly species *Cloeon dipterum* and the chironomid genus *Chironomus* (Figure S1).

During larval stage both the mayfly and dragonfly species are found swimming in the water column. *C. dipterum* is an agile fast swimmer found mostly in clear areas of the water column. *S. striolatum* is found mostly in macrophyte assemblages and is a much slower swimmer, capable of bursts of speed when attacking its prey. *Chironomus* species on the other hand are very slow and found mostly hiding in the sediment. These differences in morphology and locomotive behaviour are expected to produce different foraging strategies for the predator with each prey type. As both *C. dipterum* and its predator are fast free-swimming species, the latter displays a foraging strategy defined as ‘active capture’. *Chironomus* is much slower than its predator and its relative velocity upon encounter is likely to be negligible, corresponding to a foraging strategy defined as ‘sessile prey’. Furthermore, their preferred microhabitat likely exposes them to different changes in thermal conditions.

Individuals were collected with 500*μm* mesh nets from their ponds of occurrence based on easily distinguishable characteristics but were only identified to species level upon return from the field. Any individuals that did not correspond to these species were then excluded from the analysis (16.8% of chironomids, 8.5% of mayflies and 9.6% of dragonflies). Length-weight regressions were used (Supplementary material) to obtain individual mass from length measurements. The three chosen taxa were found in a minimum of four sites each (Table S1).

EXPERIMENTAL DESIGN

We used oxygen consumption over time as a proxy for metabolic rate (Angilletta, 2009). Measures of metabolic rates were carried out using standard respiration protocol (Supplementary material, (Brodersen *et al.*, 2008)) with Unisense O2 sensors (Unisense, Denmark). We carried out respiration trials for all three species in each of the sites at 5°C intervals from 10°C to 45°C in order to capture both the activation and deactivation energy of respiration. Water temperature was controlled using a water bath. Two trials of 7 individuals each, were carried out at each temperature value. All individuals used in these experiments were previously stored and starved in filtered pond water at ambient temperature for 24h to allow for gut clearing.

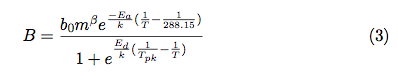
METABOLIC RATES

Temperature has inherent properties that affect species’ biology at the most basic level (Gillooly *et al.*, 2001). The metabolic theory of ecology (MTE) (Brown *et al.*, 2004) predicts that enzymatic activation energy scales linearly with the inverse of temperature following an Arrhenius function:



Where *B* is the metabolic rate, *Ea* is the enzyme’s activation energy, *k* is Boltzmann’s constant, *T* is the temperature (in *K*) and *B0* is the normalisation constant.

The Schoolfield model (Schoolfield et al., 1981) describes metabolic rate based on enzyme kinetics. This measures metabolic rate as a function of temperature including the enzyme’s temperature deactivation energy past the peak performance temperature. We used a simplified version of the model that ignores low temperature inactivation as not enough recordings were available to measure it:



Where B is oxygen consumption rate, *b0* is the normalisation constant at a reference temperature (*Tref* ), m is mass, *β* is its scaling exponent, *Ea* is the enzyme’s activation energy, *Ed* is its deactivation energy, *k* is Boltzmann’s constant, *T* is temperature and *Tpk* is the temperature at which the *B* is maximised. Each site’s median temperature was used as reference temperature (*Tref* ) to estimate a biologically relevant value for *b0*. Mass scaling was left as a free parameter to be estimated from the data after a model choice procedure was carried out (Supplementary material). All parameters were thus estimated from this function via non-linear least squares fitting of the model to the data collected for each species at each site using the ”minpack.lm” package (Elzhov *et al.*, 2016) for the statistical software R (R Core Team, 2015). Starting parameters for the fitting were sampled 10,000 times from a normal distribution centered on values estimated from equation 2 to determine the parameter values that yielded a best fit to the data (lowest AIC, BIC and highest R2).

Respiration curves were plotted for each site to test for potential adaptation patterns. Estimated parameter values were then compared between local populations at each site. Parameter values were considered statistically different from each other when associated confidence intervals did not overlap.

VELOCITY CALCULATION

Velocity is expected to scale linearly with metabolic rate (Tucker, 1970), thus, a function of basal metabolic rate was used to measure basal velocity. As oxygen consumption was used as a proxy of metabolic rate, this measurement was converted to a measure of velocity for the model designed in this paper. Oxygen, in animals, is absorbed for respiration in order to produce energy that can later be used for bodily functions. Elliott and Davison (1975) have shown that the average production of energy, via the combustion of carbohydrates, fat and protein, yields 3.34 calories per *mg* of oxygen. Oxygen consumption, measured in *μmol/h* can be converted to *g.h-1* by multiplying by the atomic mass of *O2*: 31.988 *g.mol-1*. This in turn can be turned into *cal.h-1* by using the mean oxidation value of 3.34 *cal.mg-1*. The laws of thermodynamics show that 1cal yields 4.1868*J*. This provides us with a measurement of energy produced by respiration in *J.s-1*. The energetics of animal movement, and specifically swimming, have been extensively studied for various species (Alexander, 2003; Videler and Nolet, 1990; Videler, 1993), yielding a relationship between cost of transport (*COT*) and metabolic rate (*B*). Rearranging Videler’s equation for velocity yields:



Where *COT* is expressed in *J.Nm-1*, *B* is in *J.s-1*, *m* is mass in *kg*, *g* is gravitational acceleration in *m.s-2* and *v* is speed in *m.s-1*. *COT* was calculated from Videler’s relationship (Videler, 1993):

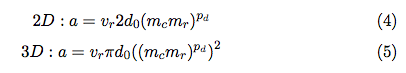


Where *m* is mass in *kg*.

SEARCH RATE MODEL

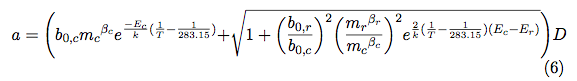
Biological rates display a unimodal response to temperature (Angilletta, 2009). Because of the biochemical processes responsible for biological traits, these rates can be modeled as an Arrhenius-Boltzmann equation (Brown *et al.*, 2004), of which equation 2 is the linearised form. As all biological rates are intrinsically determined by an individual’s metabolic rate, they are expected to follow a similar relationship to temperature (equation 3).

In biologically realistic conditions, where prey abundance in the environment does not reach predator saturation levels, handling time (*h*) can be ignored (*h = 0*) as only the dynamics of the rising, search rate dominated, part of the disk equation (Holling, 1959) will take place. Thus, the functional response curve will be dependent upon temperature with respect to *a*: the area, or volume, a predator will be able to look for a prey every second. Pawar *et al.* (2012) have shown that search rate itself is influenced in part by prey and predator traits and by the environment they interact in. The effect of dimensionality on search rate depends on whether the predator is foraging in a 2D or 3D environment and scales as follows:



Where *vr* is the relative velocity of the prey and predator, *d0* is the minimum detection distance, *mc* and *mr* are predator and prey mass respectively and *pd* is the scaling exponent of mass with dimensionality. The values of *pd* in 2D and 3D are 0.68 and 1.05 respectively (Pawar *et al.*, 2012).

For two species moving in random directions in the same environment, their relative velocity depends upon respective velocities of each species (Dell *et al.*, 2014). Velocity, as a biological rate is expected to follow equation 3 with respect to temperature dependence. The energetic power needed to put an individual in motion is provided by metabolism. Thus, the value for *b0* in equation 3 was converted to a measure of velocity in *m.s-1*. Adding equation 3 into equations 4 or 5 (as *D*) thus yields:



This model predicts the search rate of a predator foraging on an active moving prey in different environments. It can be adapted to a scenario for sessile prey by setting the prey’s velocity to 0:



There are known limitations to this model: measured metabolic rates are used as a function of velocity without taking into account other energetic needs (the whole budget goes to velocity) or increases in metabolism during activity and the efficiency of energy conversion by muscles is ignored. Nevertheless, this model is expected to provide us with a mechanistic prediction of predator search rates in various conditions and for any given species where mass and metabolic rates are known.

The model’s output was plotted for differently adapted to qualitatively test their effect on locally adapted species search rates over a gradient of temperature.

**Results**

METABOLISM BIOCHEMISTRY

Schoolfield models (equation 3) were fit to all the respiration data for the species where enough was gathered in each specific location. The best fit model out of 10,000 runs in each case was kept to estimate the values of *Ea, Ed, b0, β* and *Tpk* (Table S4).

Local species populations’ metabolic rates are expected to vary as they adapt to new environments (Kingsolver, 2009). Horizontal shift scenarios in TPC (Figure 1b) between locally adapted populations were not supported for any taxa (Figure 3). Temperature of peak performance for each taxa did not vary significantly between sites, all *Tpk* values fell within the largest confidence interval measured for a given genus (Figure 4a). Differences in TPC elevation between sites were measured for all three species (Figure 3). These changes in elevation were supported by large variations in *b0* recorded for all taxa. Populations adapted to intermediate site temperatures displayed higher *b0* values than those at each extreme (Figure 4b). Unexpectedly significant variation in *Ea* was also recorded between sites for each taxa, with colder adapted populations showing higher activation energy values (Figure 4c).

Metabolism temperature performance curves of each predator-prey pair per site were transformed, using supplementary material equation 10, into a measure of velocity and plotted against experimental temperature (Figure 5). Similar to respiration, no changes in velocity temperature performance curve *Tpk* were observed (316±6.6K and 316±7.9K for *Chironomus*, 308±1.2K and 312±1.5K for *S. striolatum*, 312±1.5K and 310±1.5K for *C. dipterum* in Evora and Toledo respectively). We find higher elevation of the prey curve relative to the predator at warmer adaptation temperatures for *Chironomus* (0.50±0.07*m.s-1* versus 0.22±0.10*m.s-1* differences in *b0*, in Evora and Toledo respectively) but not for *C. dipterum* (0.12±0.03*m.s-1* versus 0.11±0.01*m.s-1* differences in *b0*, in Evora and Toledo respectively). The predator-prey mismatch is driven by a difference in curve elevation (Figures 1c and 3), which is itself driven by a change in performance at warmer temperatures (*b0*).

SEARCH RATES AND TEMPERATURE

Expected search rates from equations 6 and 7 were plotted over a range of temperatures covering the species’ operational temperature range (Figure 6). For both species pairs and all strategies at both sites, we observe an exponential increase in search rates as expected from Boltzmann-Arrhenius scaling of biological rates. Both 2D strategies displayed the highest search rates over the OTR in all cases except for cold acclimated *C. dipterum* where 3D active prey search rates were higher than 2D sessile prey search rates. Search rates for the 3D sessile prey model were consistently smaller for both species pairs at each site.

Activation energies and elevation of all search rate curves were estimated using equation 2 (Figure 7). Activation energies of sessile search rate models remained the same for both species pairs regardless of the acclimation temperature. Diverging patterns were found for active prey strategies where activation energies (*Ea*) for *C. dipterum* search rates were higher in warm acclimated populations whilst activation energies for *Chironomus* search rates were higher in colder acclimated populations. The opposite pattern was observed for the elevation parameter (*ln(B0)*) where higher values were recorded for cold acclimated populations in *C. dipterum* but warm acclimated populations for *Chironomus*, regardless of foraging strategy model.

**Discussion**

Species-specific population temperature performance curves in this study display a vertical shift in the whole curve with increasing temperature (Figures 1c and 3). We do not observe any significant horizontal shift in the TPCs, typical of a shift in optimal performance temperature determined by trade-offs of performing better at higher temperatures and thus worse at lower ones (Figure 1b). The energy available to these species seems to increase with temperature. Yet, this observed increase is not monotonous; both high and low temperature extremes display lower elevation than intermediate temperatures. This suggests a potential optimum adaptation towards the mid-range temperatures of the OTR. Furthermore, it is worth noting that the value for *Tpk* is higher than the operational range of temperature (Figure 3). Displaying *Tpk* values at unrealistically high temperatures of 35 °C or more provides a margin of safety to these taxa should climate change bring ambient temperatures higher than they currently are.

Converting metabolic rate measurements into velocity, a key driver of species interactions (Dell *et al.*, 2014), enables us to directly consider the potential mismatch in trait performance between a prey and its predator (Figures 1d and 4). Our study suggests that species adapt to new environments within the limits imposed by their physiology and phenotypic plasticity, which will lead to differing changes in performance and thus interaction types and strengths (Angilletta, 2009; Gibert and De- Long, 2014; Kordas *et al.*, 2011; Pawar *et al.*, 2015). In this case, the predatory dragonflies of this species may be less able to feed on *Chironomus* species at higher temperatures as these may be able to move faster, and escape better, relative to their predator. On the other hand, hotter environments seem to lower *C. dipterum* performance at higher temperatures. These differences in prey response to increased temperatures could be due to the decreased oxygen concentration in warm water (?). Indeed, mayfly species are very sensitive to low *O2* environments (Bauernfeind and Soldan, 2012), making them potentially more susceptible to rises in temperature than the bottom-dwelling *Chironomus*. However, this relative change in performance only seems to be relevant past 30*°C*, thus not affecting species interactions within most of their OTR.

The set of assumptions of this model may limit its application to all types of predator-prey pairs. First, random movement has been shown to approximate animal dispersion in most cases but may not suffice in all (Pawar *et al.*, 2015). Second, the effect of dimensionality is largely dependent on the environment and the predator’s detection mechanism, as *d0* (minimum detection distance) will depend both on the medium and trait used (Pawar *et al.*, 2012). Third, the model uses an established relationship for general forms of movement in water and cost of transport (Tucker, 1970; Videler and Nolet, 1990; Videler, 1993). Alternatives can be used based on specific locomotion techniques and the physics involved therein but this was not available for our species (Alexander, 2003). We suggest that this drawback is only minor, for it is much easier to find information on higher taxa’s locomotion and work is ongoing on the physics of animal movement with regard to metabolism (Gibert *et al.*, 2016; Hein *et al.*, 2012). However, these shortcomings all point towards the fact that more empirical work on life history, habitat choice and animal movement is needed for a better integration of computational methods into biological studies. Finally, for ease of study and because no such information was available, we ignored energetic budgets and assumed that all the energy produced by metabolism was used for locomotion. This assumption will be broken in all cases as the energy produced by metabolism needs to be allocated to several other biological needs, such as growth and maintenance. Furthermore, of the energy allocated to locomotion, a large amount will be lost due to muscular inefficiency in most animals (Alexander, 2003). Indeed, up to 60% of the energy allocated for muscle contraction may be lost due to this inefficiency (Tucker, 1975). Although these assumptions are most likely affecting our results, we suggest that this model can, and should, be easily adapted for taxa where more information is available and may lead to predictions that can be validated empirically. This in turn would be hugely beneficial in studies on the effects of global warming on the dynamics of predation as search rates are notoriously complicated to estimate experimentally (Rall *et al.*, 2010; Vucic-Pestic *et al.*, 2011).

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**Authors contribution statement**

F. Affinito, M. Matias, S. Pawar and R. Kordas conceived the ideas and designed methodology;

F. Affinito, M. Matias and R. Kordas collected the data;

F. Affinito analysed the data;

F. Affinito led the writing of the manuscript.

M. Matias, S. Pawar and R. Kordas revised and corrected the manuscript.

All authors contributed critically to the drafts and gave final approval for publication.

**Supplementary Material**

LENGTH-WEIGHT REGRESSION

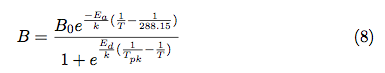
Between 50 and 100 individuals of all three tax “types”, *Odonata*, *Ephemeroptera* and *Chironomidae*, were used in each length-weight regression experiments. Each individual was measured under the microscope and placed in an individual foil cup. All cups were labeled and left in an oven at 80*°C* for 16 to 18 hours. Dry-weight measurements were then done for each individual in turn. The obtained length and biomass measurements were then fitted to two different linear models, one with dry-weight logged and not the other. The best-fit model (highest *R2*) was kept. Only *Odonata* and *Ephemeroptera* linear models yielded satisfactory fit (*R2* > 0.6) and were thus kept. The length-weight regression for *Chironomidae* was taken from (?). The equations for *Odonata* and *Ephemeroptera* and corresponding *R2* values can be found in table S2.

RESPIROMETRY PROTOCOL

All individuals selected for respirometry experiments were initially stored in filtered pond water kept at ambient temperature. These were then placed in a water bath, previously heated at the experimental temperature, for 15min to allow them an acclimation time from their ambient temperature storage to the new temperature. After acclimation, individuals were placed in glass chambers, filled with fully oxygenated filtered pond water, of 4, 2 or 0.75 *ml* depending on the size of the organism. These chambers were then placed in the respirometry apparatus inside the water bath. A total of eight chambers was used per experimental trial, one control -empty- chamber and seven treatment -organism- chambers. A Unisense O2 optical measuring probe was used to measure oxygen consumption over time in the chambers, three readings were recorded for each chamber in order to measure the slope of O2 consumption. This value was corrected for individual chamber volumes and the value of the control was subtracted from the treatment slopes to account for any respiration occurring in the chambers due to microorganisms. This slope value was then used as the value for oxygen consumption of the organism at the corresponding experimental temperature in all subsequent analysis.

RESPIRATION MODEL CHOICE

A simplified version ignoring low temperature inactivation of the mechanistic model for respiration designed by Sharpe & Schoolfield (Schoolfield *et al.*, 1981) was used to fit the respirometry data. Three variants of this model were tested for each species at each site. The model is as follows:



Where *B* is oxygen consumption rate, *B0* is the normalisation constant at 15*°C*, *Ea* is the enzyme’s activation energy, *Ed* is its deactivation energy, *k* is Boltzmann’s constant, *T* is temperature and *Tpk* is the temperature at which *B* is maximised.

The normalisation constant scales with mass as follows:



Where *m* is mass, *β* is the scaling exponent and *b0* is the normalisation constant of the Arrhenius model.

Thus, three Sharpe-Schoolfield models were run with different scalings for *b0*. One model where mass scaling was ignored (*B0* = *b0*), one where *B*0 scaled with mass according to the metabolic theory of ecology (*β* = 0.75, (Brown et al., 2004)) and one where mass scaling was left free and β was estimated from the data along with all other parameters of the model. For each species at each site, 10,000 models of each type were run, the best fit model was selected based on the overall mean fit (*R2*), AIC and BIC values of all runs (Table S3).