Temperature effects on metabolic scaling of a keystone freshwater crustacean depend on fish-predation regime

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SUMMARY STATEMENT

The metabolic pace of life of a species relates to not only body mass and temperature, but also predation regime and the interactive effects of all three of these factors.

ABSTRACT

According to the metabolic theory of ecology, metabolic rate, an important indicator of the pace of life, varies with body mass and temperature due to internal physical constraints. However, various ecological factors may also affect metabolic rate and its scaling with body mass. Although reports of such effects on metabolic scaling usually focus on single factors, the possibility of significant interactive effects between multiple factors requires further study. In this study, we show that the effect of temperature on the ontogenetic scaling of resting metabolic rate of the freshwater amphipod *Gammarus minus* depends critically on habitat differences in predation regime. Increasing temperature tends to cause decreases in the metabolic scaling exponent (slope) in population samples from springs with fish predators, but increases in population samples from springs without fish. Accordingly, the temperature sensitivity of metabolic rate is not only size-specific, but also its relationship to body size shifts dramatically in response to fish predators. We hypothesize that the dampened effect of temperature on the metabolic rate of large adults in springs with fish, and of small juveniles in springs without fish are adaptive evolutionary responses to differences in the relative mortality risk of adults and juveniles in springs with versus without fish predators. Our results demonstrate a complex interaction among metabolic rate, body size, temperature, and predation regime. The intraspecific scaling of metabolic rate with body size and temperature is not merely the result of physical constraints related to internal body design and biochemical kinetics, but rather is ecologically sensitive and evolutionarily malleable.

INTRODUCTION

The rate of biological processes or 'pace of life' has major consequences for the survival and reproductive fitness of organisms, including their ability to capture food and avoid becoming food. An important indicator of the pace of life is the rate of metabolism, which fuels all biological activities (Brown et al., 2004; Glazier, 2015). Given the central importance of metabolism in supporting the time-sensitive activities of life, biologists have devoted much attention to what controls its rate.

The influential 'metabolic theory of ecology' (MTE) focuses on two primary factors, body size and temperature (Brown et al., 2004; Sibly et al., 2012). According to the resource-transport network (RTN) model (West et al., 1997) underlying the MTE, body-size-related constraints on the supply of resources to cells via branching, space-filling transport networks cause metabolic rate (R) to scale with body mass (M) according to the simple power function

$$R = aM^b \qquad (1)$$

This equation, first used by Krogh (1916) to quantify metabolic scaling, describes a linear relationship in log-log space, where a is the scaling coefficient (antilog of the intercept of the log-linear regression line, at which M is 1), and b is the scaling exponent (slope of the log-linear regression line). The RTN model of West et al. (1997) specifically predicts that b should equal 3/4 or nearly so, thus providing theoretical support for the classical 3/4-power law (Kleiber, 1932, 1961; Hemmingsen, 1960; Savage et al., 2004; Banavar et al., 2010). In addition, the MTE assumes that increasing temperature (T) accelerates the rate of the biochemical reactions constituting metabolism in an exponential way, following the Arrhenius equation (Arrhenius, 1915; Laidler, 1984; Gillooly et al., 2001). Furthermore, the MTE assumes that M and T have independent, multiplicative effects on metabolic rate.

However, several studies have shown that M and T interactively affect metabolic rate, causing the body-mass scaling exponent b to covary significantly with T, both within and across species (reviewed in Glazier, 2005, 2014c, 2018a, 2020). Therefore, some researchers have recommended that equations describing the combined effects of M and T on metabolic rate should have an interaction term (Xie and Sun, 1990; Ohlberger et al., 2012; Glazier, 2014b). A growing number of studies have also shown that b varies in response to many other biological and ecological factors, including life style (Glazier, 2005, 2006; 2014c, 2018a, 2020; Killen et al., 2010; Pequeno et al., 2017; Tan et al., 2019), activity level (Weibel et al., 2004; Glazier, 2005, 2008, 2009a, 2010, 2020; White et al., 2007; Carey et al., 2013; Dlugosz et al., 2013), developmental stage (Wieser, 1984; Riisgård, 1998; Glazier, 2005; Killen et al., 2007; Czarnołęski et al. 2008; Glazier et al., 2015), growth rate (Glazier, 2005, 2014b; Czarnołęski et al. 2008; Glazier et al., 2011; Tan et al., 2019) and predation regime (Glazier et al., 2011; Gjoni et al., 2020), among others (reviewed in Glazier, 2005, 2014b, c; White and Kearney, 2014).

Most studies of the ecology of metabolic scaling have focused on the effects of single environmental factors, each considered alone. Although several studies have examined the combined effects of temperature and various chemical or physical factors on the metabolism of aquatic animals (e.g., Mosser and Hettler, 1989; Claireaux and Lagardère, 1999; Finn et al., 2002; Spanopoulos-Hernández et al., 2005; Allan et al., 2006; Glover et al., 2012; Carey et al., 2016; Leiva et al., 2018; Kratina et al., 2019; Schwieterman et al., 2019; and references therein), only a few have reported size-specific, interactive environmental effects on the metabolic scaling exponent (Dehnel, 1960; Newell et al., 1972; Carey & Sigwart, 2014). Our study (along with accompanying work by Gjoni et al., 2020: see the Discussion section) is the first to show that an abiotic environmental factor (T) and a biotic environmental factor (predation regime)

interactively affect metabolic scaling. These results have both scientific and applied value. First, they not only reinforce the view that the body-mass scaling of metabolic rate is highly sensitive to many kinds of external (ecological) factors and not simply the result of internal body-design constraints, as assumed by the MTE, but also show that specific ecological effects may themselves depend on other interacting ecological factors. Second, our findings extend the wide range of reported top-down effects of predators on prey traits to include the temperature-sensitive scaling of metabolic rate with body mass. Several studies have examined effects of predators on prey metabolic rates (reviewed by Glazier et al., 2020; also see Jermacz et al., 2020a, b), but few have focused on how predators affect metabolic scaling (Glazier, 2006; Glazier et al., 2011; Gjoni et al., 2020). Moreover, our work shows that predator effects on prey are temperature sensitive, not only with regard to metabolic rate (e.g., Janssens et al., 2015; Laws, 2017), but also its scaling with body mass. Gjoni et al. (2020) have examined how temperature and chemical cues of fish predators interactively affect metabolic scaling, whereas the present study examines interactive effects of temperature and the predation regime of source habitats. Metabolic scaling is not a simple result of bottom-up effects of resource supply, as assumed by the MTE, but also is influenced by top-down effects on resource-demanding processes, such as growth and behavioral activity (also see Glazier et al., 2011, 2020; Harrison, 2017; Hatton et al., 2019). Third, our results suggest that effects of climate (T) change on biological systems may not be simple results of biochemical kinetics, as assumed by the MTE, but may depend critically on the ecological context.

In the laboratory, we investigated the effects of temperature on the ontogenetic metabolic scaling of mid-Appalachian populations of the amphipod *Gammarus minus* inhabiting three freshwater springs with fish predators and three without. Amphipods are abundant keystone

species in many springs, streams and groundwater ecosystems throughout temperate regions of the world (Glazier, 2014a). Their advantages for metabolic scaling studies include ease of collection and study throughout the year, lack of postembryonic developmental metamorphosis (thus simplifying ontogenetic comparisons of metabolic rate), and a body-mass range exceeding 30-fold, thus ensuring adequate power of the regression analyses (also see Methods). The study springs are also advantageous because they have similar, relatively constant water temperatures and chemical compositions, and their macroinvertebrate faunas consist mostly of amphipods (*G. minus)* and relatively few other kinds of species, thus representing useful, relatively simple, naturally controlled ecosystems for studying a wide variety of ecological and evolutionary questions (cf. Glazier, 2014d). In addition, the study populations of *G. minus* are relatively isolated geographically, thus allowing adaptive responses to differences in predation regime to evolve in the absence of substantial gene flow. Mid-Appalachian populations of *G. minus* exhibit significant genetic differentiation (Gooch, 1990; Gooch and Glazier, 1991; Kane et al., 1992; Carlini et al., 2009) and the study populations, in particular, display significant morphological, behavioral and life-history differences between springs with versus without fish predators (Glazier, 1999, 2000; Glazier and Deptola, 2011; Glazier et al., 2011, 2020; Glazier and Paul, 2017).

MATERIALS AND METHODS

Sample sites

The study springs in Blair and Huntingdon Counties of central Pennsylvania (USA) have similar physical and chemical conditions (i.e., clear, shallow, cool, well oxygenated, moderately flowing hard water; common occurrence of watercress, *Nasturtium offinale*; temperature ≈ 9-13º C; pH ≈ 6.6-7.7), but three contain the predatory fish *Cottus cognatus* (Ell, Blue and Williamsburg) and three do not (Petersburg, Kanesatake and Big Rock). Amphipods are a major component of the diet of *C. cognatus* (Newman and Waters, 1984) and other *Cottus* species (MacNeil et al., 1999). Other predators in the study springs are either rare (e.g., salamanders) or relatively ineffective at preying on healthy amphipods (e.g., flatworms) (Jenio, 1979; Glazier and Gooch, 1987). The macroinvertebrate faunas of the study springs are relatively species poor with the most abundant species including the amphipod *G. minus* at all sample sites, and at some sites also the isopod *Lirceus brachyurus* (Blue Spring) and the hydrobiid snail *Fontigens nickliniana* (Ell, Williamsburg and Kanesatake Springs). Further details about the study springs can be found in Glazier and Gooch (1987), Glazier et al. (1992), Glazier (1999), Glazier et al. (2011), and Glazier and Paul (2017).

Amphipod collection, temperature acclimation and measurements of metabolic rate

Amphipods with a wide range of sizes (~0.06 to 8 mg dry body mass) were collected with dip nets during the spring, summer and fall months of 2010, 2018 and 2019, and acclimated in 5-L basins containing aerated water and natural detrital leaf food from their native spring at one of three temperatures (4, 10 and 16º C) in an environmental control room for at least seven days

before their metabolic rate was measured. The normal mean temperature of our study springs throughout the year is near 10º C, which we used as a reference point for choosing symmetrically lower and higher experimental temperatures at levels causing minimal mortality during acclimation. Before a respirometry run, experimental amphipods (totaling 710 individuals) were fasted for 24 h (to remove the metabolic effects of feeding) in porous 120-mL cups placed in a 5-L basin of aerated native spring water at each of the experimental temperatures (4, 10 and 16º C). During starvation, a gammarid gut is evacuated within 24 h at temperatures ranging from 5 to 15º C (Marchant and Hynes, 1981). Each coprophagous amphipod was placed in the upper compartment of each cup separated by nylon mesh from the lower compartment where feces could collect without being eaten.

We measured the oxygen consumption rate (a proxy for metabolic rate) of individual amphipods using a Strathkelvin respirometry system (following Glazier and Sparks, 1997; Glazier et al., 2011). Adults were only males to avoid possible confounding effects of eggs or embryos carried by females on metabolic rate (see Glazier, 1991; Leiva et al., 2018). The respirometers were glass syringes containing native spring water filtered with Whatman GF/F 0.7-µm glass filters to remove metabolically active microbes. Syringe size (2, 5, or 10mL) and water volume varied with size of the amphipod (1-4, 3-6, 7-10+mm body length, respectively), so that the oxygen concentration never dropped below 63% saturation (and usually not below 75% saturation) during 6 h of incubation. To estimate resting metabolic rates (*RMR*), amphipod movement and associated energy expenditure were minimized by making respiration measurements during the day (9:00 to 18:00) when amphipods are least active (cf. Elliott, 2002; Peeters et al., 2009), and by placing pieces of 0.3-mm nylon mesh in each relatively small confining syringe to permit clinging behavior and to restrict the space in which amphipods could

move (following Glazier et al., 2011; also see the Discussion section). The blunted needle of each syringe was plugged with melted wax. Care was taken to remove all air bubbles before incubation. Controls were without amphipods (one control per 4-8 experimental syringes). We omitted data for amphipods that molted during a respirometry run because molting in amphipods and other arthropods increases metabolic rate above normal resting levels (Sutcliffe, 1984; Glazier, 2005). Oxygen concentrations of 50-μL water samples were measured by injecting them through each syringe needle into a Strathkelvin microcell containing a 1302 oxygen electrode connected to a Strathkelvin Model 781 oxygen meter (Strathkelvin Instruments, Glasgow, UK). Rate of oxygen consumption (R_{O2}, mL O_2/h) was calculated as

$$R_{O2} = [(P_E - P_C)\,S \times A \times V]/t$$

where P_E is the partial pressure (1 Torr ≈ 1 mm Hg) of oxygen in an experimental syringe, P_C is the partial pressure of oxygen in a control syringe, S is the solubility coefficient of oxygen in water (2.57, 2.22 and 1.82 μmol L^{-1} $Torr^{-1}$ at 4, 10 and 16º C, respectively), A is the volume of 1 mol O_2 at standard temperature and pressure (22.414 L/mol), V is the volume of water in a syringe (liters), and t is the incubation time (h). Immediately after a respiration rate measurement was made, each amphipod was stored at -70º C, and to determine dry body mass, dried in an oven at 60º C for 60 h, and weighed (±l μg) on a Cahn C-31 microbalance (Cahn Instruments, Cerritos, California, USA).

Statistical analyses

We used least squares regression (LSR) to relate metabolic rate to body mass (following Glazier et al., 2011). This method is preferred over reduced major axis (RMA) analyses when the independent variable (body mass) is measured with much less error than the dependent variable

(metabolic rate) (Smith, 2009), as is typically the case for metabolic scaling relationships (Glazier, 2010, 2014b; White, 2011). Log$_{10}$-transformation normalized the data variation, and permitted proportional, linear relationships to be readily discerned (Kerkhoff and Enquist, 2009; Glazier, 2013). The significance of differences among scaling exponents (slopes) and intercepts was estimated by ANCOVA (with body mass as a covariate), and by comparing these parameters using 95% confidence intervals. If a mean value was outside the 95% CI of another mean value and vice versa, they were considered significantly different ($P < 0.05$), following Smith (1997). We used SYSTAT 10 software (SPSS Inc., Chicago, Illinois, USA) for all statistical analyses.

We deemed the sampled body-mass ranges as sufficient for statistical testing because they were greater than the one order of magnitude often used as the minimal acceptable range in experimental and comparative studies of intraspecific metabolic scaling (see e.g., Xie and Sun 1990; Glazier 2009b, 2020; Vaca and White, 2010; Carey et al., 2013; Hirst et al., 2014; Fossen et al., 2019; Tan et al., 2019). In addition, they were similar to those used in other amphipod studies that successfully detected significant population or species differences in the scaling of metabolism and other traits (e.g., Glazier et al., 2011; Glazier & Deptola, 2011; Glazier and Paul, 2017; Shokri et al., 2019; Gjoni et al., 2020; Glazier et al., 2020).

RESULTS

Individual variation in resting metabolic rate (*RMR*) of *Gammarus minus* was large, as often reported in other studies of amphipods (e.g., Sutcliffe, 1984; Glazier et al., 2011; Galic and Forbes, 2017; Semsar-kazerouni and Verberk, 2018; Kratina et al., 2019; Shokri et al., 2019; Gjoni et al., 2020), and other animals (e.g., Phillipson and Watson, 1965; Kremer, 1975; Chown et al., 2007; Careau et al., 2008; Gudowska et al., 2017; also see the Discussion section).

Nevertheless, we found highly significant relationships between *RMR* and body mass (*M*) at all three experimental temperatures for aggregate amphipod samples from the three springs with fish predators and the three without fish predators (Tables 1 and 2; Figs. 1 and 2). Significant relationships between *RMR* and *M* also occurred in all individual population samples, except for the Williamsburg Spring population samples at 10 and 16°C (Tables 3 and 4; Fig. 3). Lower correlation coefficients appear to be related to smaller available body-mass ranges in populations exposed to size-selective fish predation (Table 3; Figs. 1, 2 and 3), especially in Williamsburg Spring where fish predators are most numerous (Glazier et al. 2011), and where it is difficult to find amphipods, most of which hide in the sediment. Stubbington et al. (2017) describe the difficulty of sampling all size classes of amphipods in sediments.

Significant effects of temperature on *RMR* occurred for both aggregate samples from the springs with fish predators and those without (Table 2) and for all six populations, as well (Table 4). However, the *RMR* responses to cooling (10 to 4°C) and warming (10 to 16°C) differed significantly between the aggregate samples from springs with versus without fish predators. Although their body-mass adjusted $\log_{10}$ *RMR* means (± 95% C.I.) did not differ significantly at the baseline native temperature of 10°C (-0.473 ± 0.082 versus -0.393 ± 0.088), they did differ significantly at 4°C (-0.346 ± 0.078 versus -0.782 ± 0.083) and 16°C (0.062 ± 0.069 versus -0.345 ± 0.077) (based on the ANCOVA analysis in Table 2; also compare Figs. 1A, 1B and 1C). Furthermore, the *RMR* scaling exponents (*b*) differed significantly with temperature (*T*) in the aggregate sample from springs without fish predators, as indicated by a significant *T* x *M* interaction, though this was not the case for the aggregate sample from springs with fish predators (Table 2). Four individual population samples (all three of the population samples from springs without fish predators, and one population sample from a spring with fish) showed

significant $T \times M$ interactions, whereas two population samples from springs with fish predators did not (Table 4). In addition, the effect of T on the elevation of the RMR scaling relationships varied among the various populations and with predation regime (Figs. 2 and 3). For example, the RMR of amphipods from springs with fish predators increased more substantially between 10 and 16º C than between 4 and 10º C, but the opposite occurred for amphipods from springs without fish predators (Fig. 2). Other kinds of inter-population variation in T effects also occurred, including unexpected decreases in overall RMR with increasing T in two populations (4 to 10º C in the Ell Spring population: Fig. 3A; and 10 to 16º C in the Big Rock Spring population: Fig. 3F).

Although b tended to decrease with increasing T in the populations from springs with fish predators (Ell, Blue and Williamsburg: Figs. 2A, 3A-C, and 4A), the opposite pattern was observed in populations from springs without fish predators (Petersburg, Kanesatake and Big Rock: Figs. 2B, 3D-F and 4B). Furthermore, at the lowest experimental temperature (4º C), populations from springs with fish predators showed significantly steeper RMR scaling relationships than those of populations from springs without fish predators (Figs. 1A and 4), whereas the opposite was observed at the highest temperature (16º C) (Figs. 1B and 4). As a result, in populations from springs with fish, small juveniles tended to show large increases in RMR with increasing T, whereas large adults showed less change (Figs. 2A and 3A-C). In contrast, in populations from springs without fish, large adults tended to exhibit large increases in RMR with increasing T, whereas small juveniles showed less change (Figs. 2B and 3D-F).

DISCUSSION

Interactive effects of temperature and predation regime on metabolic scaling

Both temperature and predation regime affected the scaling of metabolic rate in populations of the amphipod *Gammarus minus* (Tables 1, 2, 3 and 4; Figs. 1, 2, 3 and 4). Furthermore, the effect of T on metabolic scaling differed markedly between populations from springs with versus without fish. As a result, at 4°C, *RMR* scaling exponents (*b*) were always higher in populations from springs with versus without fish predators (0.718 to 0.912 versus 0.440 to 0.575), whereas the opposite occurred at 16°C (0.374 to 0.612 versus 0.923 to 1.007: see Fig. 4).

These results challenge various theoretical models of metabolic scaling. For example, they contradict the MTE, which assumes that *b* is fixed and unrelated to T and other environmental factors (Brown et al., 2004). The cell-size model (Davison, 1955; Kozłowski et al., 2003) and classical Dynamic Energy Budget (DEB) theory (Kooijman, 2000) do not consider effects of T and predators on metabolic scaling, though potentially they could be modified or extended to do so. Only the metabolic-level boundaries hypothesis (MLBH) (Glazier, 2005, 2010, 2014c; Killen et al., 2010), the viscosity hypothesis (VH) (Verberk and Atkinson, 2013), the acclimation hypothesis (AH) (Fossen et al., 2019), and a modified version of DEB theory applied to some colonial animals (White et al., 2011; Kearney and White, 2012; cf. Glazier 2018a) predict that T should affect metabolic scaling. According to the MLBH, the *RMR* scaling exponent should correlate negatively with the overall level of metabolism, as indicated by the elevation of a scaling relationship (Glazier 2005, 2008, 2010). This should occur because volume-related tissue demand should chiefly influence *RMR* at low metabolic levels (*b* approaching 1), but surface-related resource supply and waste removal should chiefly influence *RMR* at high

metabolic levels (b approaching 2/3 in isomorphic organisms). Therefore, since increasing T increases the overall level of RMR, b should correlate negatively with T, as often observed in ectothermic organisms (Glazier, 2005, 2014c, 2018a), and especially in sedentary species (Glazier, 2020). The VH and AH also predict a negative relationship between b and T. However, although the inverse correlations between b and T observed in amphipod populations from springs with fish predators (Figs. 2A, 3A, 3B, 3C, 4A) are consistent with the MLBH, VH and AH, the positive correlations between b and T observed in populations from springs without fish (Figs. 2B, 3D, 3E, 3F, 4B) seem to contradict all of these hypotheses. However, as supported by evidence reported by Glazier (2020), the MLBH predicts that positive associations between b and T may arise because increasing T may increase various volume-related, energy-demanding processes such as growth and activity, thus causing b to increase toward isometry. The specific hypothesis proposed below follows this line of reasoning.

Hypothetical explanation based on size-specific variation in predation risk

We propose a novel working hypothesis to explain why predation regime adaptively alters the effect of T on the metabolic scaling of *G. minus* populations (Fig. 5). Although this hypothesis focuses only on our specific results, one may modify it so that it may potentially apply to other species with various patterns of age- and size-specific mortality in diverse ecological contexts. Our specific hypothesis relies on two well-supported assumptions. First, it assumes that visually hunting fish (*Cottus cognatus*) are more of a threat to large conspicuous amphipods than smaller less visible amphipods. Four observations support this assumption. First, large amphipods occur more frequently in the guts of fish predators (including *C. cognatus*) than in the surrounding habitat (Newman and Waters, 1984; Allan and Malmqvist, 1989). Second, the relative frequency

of small versus large amphipods is higher in our study springs that contain fish predators versus those that do not (Glazier et al., 2011). Third, in the presence of fish predators, large adult amphipods tend to reduce their behavioral activity more than do smaller juvenile amphipods (Newman and Waters, 1984; Andersson et al., 1986; Allan and Malmqvist, 1989; Friberg et al., 1994). Fourth, large adult amphipods tend to have significantly lower fat levels in springs with versus without fish predators, whereas smaller juvenile amphipods do not show this difference or an opposite effect (Glazier et al., 2011; Glazier et al., 2020). This size-specific pattern is likely due to reduced foraging activity by large conspicuous amphipods in springs with fish. Many studies have shown that the presence of fish predators reduces feeding activity in amphipods (e.g., Newman and Waters, 1984; Friberg et al., 1994; Åbjörnsson et al., 2000; Beermann et al., 2018).

Second, our hypothesis assumes that, given the need or opportunity, large amphipods will cannibalize smaller, more vulnerable amphipods. Amphipods are opportunistic omnivores that often engage in size-specific cannibalism (Dick, 1995; MacNeil et al., 1997, 1999; Lewis et al., 2010). Cannibalism should be especially severe on small juvenile amphipods when large adults are numerous and likely also highly voracious, because of the depletion of other food resources at high population densities. Amphipod cannibalism increases when other food resources are not available (Dick, 1995). The presence of large adult amphipods can also alter the behavior of smaller juvenile amphipods, apparently as an anti-predation response (MacNeil et al., 1997, 1999; McGrath et al., 2007).

Based on these assumptions, we propose that habitat differences in predation regime have caused adaptive evolutionary, size-specific shifts in the responses of metabolic rate to changes in temperature. According to our hypothesis (Fig. 5), in populations inhabiting fish springs, young

small inconspicuous amphipods may significantly increase their activity and growth (and thus metabolic rate) in response to increasing T without significantly increased mortality from fish predators, whereas older larger, more conspicuous amphipods cannot do this without suffering heavy mortality. As a result, these size-specific, adaptive responses in metabolically costly activity and growth cause the metabolic scaling lines at different temperatures to converge at larger body sizes.

However, in populations from fishless springs, large adult amphipods may significantly increase their activity and growth (and thus metabolic rate) in response to increasing T without increased mortality risk, whereas younger smaller amphipods cannot do this without suffering substantial mortality from numerous actively foraging cannibalistic adults (made possible by absent fish predation). Therefore, in fishless spring populations, the metabolic scaling lines at different temperatures tend to converge at smaller body sizes. In short, relative mortality on juveniles versus adults may drive size-specific, energy-expensive growth rates and activity levels, and thus the rate of metabolism supporting them.

Our hypothesis (Fig. 5) is applicable to not only field metabolic rates, but also resting metabolic rates observed in the laboratory. This is because the cost of growth contributes significantly to the resting metabolic rate of ectothermic animals with low maintenance requirements (Parry, 1983; Wieser, 1994; Glazier, 2015; Glazier et al., 2020). In addition, although we attempted to minimize activity during our respiration measurements, we could not eliminate it altogether, and thus low levels of spontaneous activity may have contributed somewhat to the metabolic rates that we estimated, a hypothesis that we are currently testing. Amphipods exhibit high levels of inter-individual variation in behavioral activity (Peeters et al., 2009; Augusiak and Van den Brink, 2015), which may have affected the metabolic rates that we

measured. Accordingly, size-specific thermal effects on activity (and growth) may underlie the shifts in metabolic scaling that we have observed. In support, Glazier (2014c, 2020) has provided evidence that temperature and activity can interactively affect intraspecific metabolic scaling in ectothermic animals.

In short, we hypothesize that conservative metabolic rates showing low sensitivity to increases in T (as in large adults in springs with fish, and small juveniles in springs without fish, but with many cannibalistic adults) are adaptive evolutionary responses to size-specific mortality. Conservative energy expenditures may also be advantageous when predators inhibit foraging activity, and thus energy intake (Wellborn, 1994; Handelsman et al., 2013; Glazier et al., 2020), as has been observed in amphipods exposed to fish predators or their chemical cues (e.g., Friberg et al., 1994; Åbjörnsson et al., 2000; Beermann et al., 2018).

Various processes related to resource uptake and use (rates of food-energy assimilation, growth and metabolism, and oxygen-uptake capacity, as indicated by gill surface area) show parallel differences in ontogenetic body-mass scaling between *G. minus* populations from springs with versus without fish predators (Glazier et al., 2011; Glazier and Paul, 2017; Glazier et al., 2020). Multi-generational exposure of *G. minus* populations to fish predators has led to similar decreases in the scaling exponents of all of the above energetically significant traits. Therefore, further understanding may result from holistically examining the interactive effects of temperature and predation regime on the whole-organism energetic economy of spring-dwelling populations of *G. minus*.

Effects of fish predators on the metabolic scaling of amphipods may be the result of not only adaptive (genotypic) evolution, but also phenotypically plastic responses. It is possible that amphipods increase/decrease their metabolic rate physiologically as an anti-predator stress

response (see Hawlena and Schmitz, 2010), and/or in anticipation of increased/decreased activity (Glazier et al., 2020). In support of immediate phenotypic effects, Gjoni et al. (2020) have shown that fish kairomones (chemical cues) significantly affected the temperature-sensitive, ontogenetic body-mass metabolic scaling of two populations of amphipods in habitats containing fish predators, *G. minus* from Blue Spring, and *G. insensibilis* from an Italian saltwater lagoon. This companion study has revealed that the metabolic scaling exponent (b) decreases at higher T in the presence of predator cues, but not in their absence. This result suggests that predation risk causes large conspicuous adults to resist T-related increases in RMR more than that of smaller less conspicuous juveniles, which parallels the apparent adaptive responses shown by *G. minus* populations from springs with fish predators, as reported in the present study. Currently, we are studying whether these phenotypically plastic effects depend on the history of exposure of amphipods to fish predators in their native habitats.

Relevance of ecological variation in intraspecific metabolic scaling to metabolic scaling theory

Most studies showing effects of ecological factors on metabolic scaling have examined intraspecific relationships, rather than interspecific relationships that are the chief focus of the MTE (but see e.g., Glazier, 2010, 2014c, 2018a; Pequeno et al., 2017). Brown et al. (2004) originally presented the MTE as a broad-scale view of biological scaling relationships based on body-mass ranges encompassing many orders of magnitude and many kinds of species (also see Sibly et al., 2012). Intraspecific scaling relationships often involve relatively narrow body-mass ranges that may be more prone to statistical error and the effects of various idiosyncratic extraneous factors than are broader scale interspecific relationships. Some investigators have further claimed that different factors or mechanisms affect intra- versus interspecific metabolic

scaling relationships (e.g., Heusner, 1982; Wieser, 1984; Kooijman, 2000; Maino and Kearney, 2014). Therefore, one might argue that studies of intraspecific metabolic scaling are not relevant to the MTE and the theory underlying it. However, we disagree with this view for four major reasons (also see Glazier, 2006). First, Kleiber (1961), a founder of the 3/4-power law upon which the MTE is based, regarded this law as applicable to both intra- and interspecific metabolic scaling. Second, proponents of the MTE have argued that the RTN model underlying this theory (West et al., 1997) is universally applicable, applying to metabolic scaling relationships both within and among species (Brown et al., 1997). Indeed, growth models developed by proponents of the MTE assume that intraspecific metabolic scaling relationships obey RTN theory, as do interspecific relationships (e.g., West et al., 2001; Hou et al., 2008; Moses et al., 2008). Third, although intraspecific scaling relationships often include relatively narrow body-mass ranges encompassing less than an order of magnitude, thus increasing the error of their estimated slopes and intercepts (e.g., Bokma 2004; Moses et al. 2008; Gudowska et al., 2017), many are based on large body-mass ranges encompassing multiple orders of magnitude (e.g., numerous kinds of animals and plants with indeterminate growth: see e.g., Wesemeier, 1960; Paine, 1971; Larson, 1987; Bokma 2004; Killen et al., 2007; Moran and Wells, 2007; Peng et al., 2010). Fourth, another major mechanism underlying the MTE, i.e., universal physical kinetic effects of temperature on the rates of various biological processes, should also operate similarly within and among species (Clarke, 2004; Gillooly et al., 2006; Dell et al., 2011).

Therefore, we believe that studies of intraspecific metabolic scaling are useful not only for specifically evaluating the MTE, but also for testing metabolic scaling theory in general. Important advantages of studies of intraspecific metabolic scaling include that they are less

affected by phylogenetic complications that plague interspecific analyses because they involve single populations or comparisons of conspecific populations that are genetically more similar than are different species or higher taxa (Bokma, 2004; Glazier, 2005); and they are more readily amenable to incisive experimental tests and comparative analyses involving individuals or populations exposed to different biological or environmental conditions (Glazier, 2006; Vaca and White, 2010; Glazier et al., 2011).

Causes of high inter-individual variability in metabolic rate

Additional research is also required to understand why metabolic rate varied greatly among individual amphipods in our study, even after accounting for the effects of body size, temperature and predation regime. Unmeasured intrinsic and extrinsic factors, such as individual differences in activity, growth and body condition, varying environmental effects occurring during the ontogeny of individuals in their natural habitats, and varying plasticity of individual responses to environmental manipulations in the laboratory may all have contributed to the high individual variation in metabolic rate that we observed. We suspect that variable levels of activity and growth are major causes of this variation (also see Peeters et al., 2009; Glazier et al., 2011; Glazier, 2020; Glazier et al., 2020), but many other phenotypic and genotypic causes are also possible. Metabolic rate is a complex trait, composed of many underlying biochemical and physiological processes, involving numerous kinds of regulatory control and varying sensitivities to environmental factors (Darveau et al., 2002; Clarke, 2004; Suarez and Darveau, 2005; Glazier 2005, 2014b; Pettersen et al., 2018). Therefore, it should not be surprising that metabolic rate varies substantially within not only amphipod species (also see the Results section), but also many other animal species (Speakman et al., 2004; Careau et al., 2008; Burton et al., 2011; Konarzewski and Książek, 2013;

Norin and Metcalfe, 2019). The phenotypic plasticity of metabolic rate in response to environmental changes may also vary immensely among individuals (Norin and Metcalfe, 2019), as observed for temperature changes in amphipods (Réveillon et al., 2019) and insects (Terblanche et al., 2007). Given the central importance of metabolism for the functioning and evolutionary fitness of organisms, understanding its intraspecific variation is now becoming a major focus of research by many kinds of biologists (Careau et al., 2008; Burton et al., 2011; Konarzewski and Książek, 2013; Pettersen et al., 2018; Mathot et al., 2019; Norin and Metcalfe, 2019).

CONCLUSIONS

Our results add further support to the view that the body-mass scaling of metabolic rate is not simply the result of internal body-design constraints on resource supply, as assumed by the MTE, but is highly responsive to many kinds of external (ecological) factors that affect resource demand. The MTE assumes that resource supply dictates metabolic rate, which in turn acts as a pacemaker for other biological and ecological processes (Brown et al., 2004). In contrast, our results support an alternative (or complementary) view – an ecological theory of metabolism (ETM) – that focuses on how ecological factors influence various kinds of resource demand, which in turn modulate the rate of supporting metabolism and its scaling with body mass (Glazier, 2006, 2014b, 2015; Glazier et al., 2011; Harrison, 2017; Hatton et al., 2019). Both resource demand and supply may affect metabolic scaling (Glazier, 2014b, 2018b, c). This view is consistent with modern theory concerning the regulation of biochemical pathways, which posits that both resource supply and demand control the rates of metabolic reactions. Much evidence supports this view (reviewed in Glazier, 2015). In addition, our results add to growing evidence that specific ecological effects on metabolic scaling may themselves depend on other

interacting biological and ecological factors. Abiotic environmental factors (e.g., temperature) may affect metabolic scaling interactively with not only other abiotic environmental factors (e.g., salinity: Dehnel, 1960; acidity: Carey & Sigwart, 2014; and exposure to air versus water: Newell et al., 1972) and intrinsic biological factors (e.g., nutrition: Newell et al., 1976; activity level: Glazier, 2014c, 2018a, 2020; and mode of thermoregulation: Glazier, 2018a), but also with biotic environmental factors (e.g., predation regime), as we show in this study (also see Gjoni et al., 2020).

Furthermore, our findings demonstrate that relationships between metabolic rate and T are not the simple result of biochemical kinetics, as assumed by the MTE (also see Schulte, 2015; DeLong et al., 2017; Moffett et al., 2018; Norin and Metcalfe, 2019; Réveillon et al., 2019). Predation regime has such a powerful effect on metabolic rate that it can significantly dampen its response to increasing T (as in adult *G. minus* from springs with fish predators: Figs. 2A, 3A-C and 5; and juveniles from springs with many cannibalistic adults made possible by the absence of fish: Figs. 2B, 3D-F and 5). Simple biochemical kinetic T effects also cannot explain why amphipods from springs with fish predators show significantly different responses to cooling (10 to 4º C) and warming (10 to 16ºC) than do amphipods from springs without fish predators (see Results section and Fig. 2). In addition, other inter-population differences in T effects (including absent or even reversed effects where *RMR* decreased with increasing T: Figs. 2 and 3) defy expectations based on biochemical kinetic models. Minimal or reversed effects of T on metabolic rate occur over various (not just upper) T ranges in other amphipod species (Issartel et al., 2005; Foucreau et al., 2014; Galic and Forbes, 2017; Semsar-kazerouni and Verberk, 2018; Kratina et al., 2019; Jermacz et al., 2020a) and many other kinds of animals, as well (e.g., Vernberg, 1959; Dehnel, 1960; Newell and Northcroft, 1967; Newell et al., 1976; Penick et al.,

1998; Seibel et al., 2007; Marshall and McQuaid, 2011; Watson et al., 2014; Thyrring et al., 2015; Harianto et al., 2018; Cooper et al., 2019; Sargent, 2019; Scheffler et al., 2019; da Silva Vianna et al., 2020; and references cited therein). Whether these thermal responses are adaptive or stress related requires further research (Schulte, 2015; DeLong et al., 2017).

Ecological effects on the intraspecific scaling of metabolic rate with body mass and temperature deserve more consideration in theoretical models, most of which focus on the internal anatomy, physiology and biochemistry of organisms. In addition, our results have important implications for understanding the effects of climate (T) change on biological and ecological systems. Biological effects of global warming may not be simple emergent functions of biochemical kinetics, but may depend critically on the ecological context. As a result, predicting biological and ecological effects of global warming becomes complicated (also see Tseng and O'Connor, 2015; Lau and terHorst, 2019; Luhring et al., 2019; Tseng et al. 2019; Truong et al., 2020). As shown in our study, warming may increase the metabolic rates of small juveniles more than that of large adult amphipods in springs with fish predators, but do the opposite in springs without fish. These habitat-related differences in how warming affects the metabolic rate (and associated biological processes) of an abundant keystone species may have important consequences for the dynamics and structure of populations and communities in springs and other aquatic ecosystems. Our results and the associated hypothetical explanation that we have proposed allow us to make three testable predictions. First, warming should accentuate harmful direct and indirect effects of fish predators (i.e., increased mortality and risk-reducing inhibition of feeding, growth and metabolism) on large adult amphipod prey in springs with fish predators, but in contrast accentuate harmful effects of cannibalistic adult amphipods on small juveniles in springs without fish predators. Second, warming should affect amphipod

population dynamics and structure differently in habitats with versus without fish predators. For example, warming should induce higher rates of juvenile growth and maturation (and thus higher rates of production of new breeding adults) in populations inhabiting springs with versus without fish predators. In addition, warming should cause the size-structure of amphipod populations to become more skewed toward smaller body sizes in springs with versus without fish predators. Third, warming should increase litter decomposition (detrital leaf shredding) rates by adult amphipods (and thus rates of nutrient cycling and loss of coarse detrital food) more in springs without versus with fish predators. As a result, greater competition for coarse particulate detrital food in detritivore communities, greater challenges to maintaining energy balance in amphipods and other shredding detritivores, and greater availability of dissolved nutrients and fine particulate food for other biota should occur in springs without versus with fish predators. However, these predicted outcomes may be altered by other ecological factors not considered here. As we have emphasized, the biological effects of warming may depend critically on the ecological context.

DATA AVAILABILITY
Data on metabolic rate in relation to body mass of the amphipod *Gammarus minus* in six populations are available in Table S1 of the Supplementary Material.

REFERENCES

Åbjörnsson, K., Dahl, J., Nyström, P. and Brönmark, C. (2000). Influence of predator and dietary chemical cues on the behaviour and shredding efficiency of *Gammarus pulex*. *Aquatic Ecol.* **34**, 379-387. doi.org/10.1023/A:1011442331229

Allan, E. L., Froneman, P. W. and Hodgson, A. N. (2006). Effects of temperature and salinity on the standard metabolic rate (SMR) of the caridean shrimp *Palaemon peringueyi*. *J. Exp. Mar. Biol. Ecol.* **337**, 103-108. doi.org/10.1016/j.jembe.2006.06.006

Allan, J. D. and Malmqvist, B. (1989). Diel activity of *Gammarus pulex* (Crustacea) in a south Swedish stream: comparison of drift catches vs baited traps. *Hydrobiologia* **179**, 73-80. doi.org/10.1007/BF00011931

Andersson, K. G., Brönmark, C., Herrmann, J., Malmqvist, B., Otto, C. and Sjörström, P. (1986). Presence of sculpins (*Cottus gobio*) reduces drift and activity of *Gammarus pulex* (Amphipoda). *Hydrobiologia* **133**, 209-215. doi.org/10.1007/BF00005592

Arrhenius, S. (1915). *Quantitative Laws in Biological Chemistry*. London, UK: Bell.

Augusiak, J. and Van den Brink, P. J. (2015). Studying the movement behavior of benthic macroinvertebrates with automated video tracking. *Ecol. Evol.* **5**, 1563-1575. doi.org/10.1002/ece3.1425

Banavar, J. R., Moses, M. E., Brown, J. H., Damuth, J., Rinaldo, A., Sibly, R. M. and Maritan, A. (2010). A general basis for quarter-power scaling in animals. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 15816-15820. doi.org/10.1073/pnas.1009974107

Beermann, J., Boos, K., Gutow, L., Boersma, M. and Peralta, A. C. (2018). Combined effects of predator cues and competition define habitat choice and food consumption of amphipod mesograzers. *Oecologia* **186**, 645-654. doi.org/10.1007/s00442-017-4056-4

Bokma, F. (2004). Evidence against universal metabolic allometry. *Funct. Ecol.* **18**, 184-187. doi.org/10.1111/j.0269-8463.2004.00817.x

Brown, J. H., Enquist, B. J. and West, G. B. (1997). Allometric scaling laws in biology. *Science* **278**, 373. doi: 10.1126/science.278.5337.369c

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. and West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology* **85**, 1771-1789. doi.org/10.1890/03-9000

Burton, T., Killen, S. S., Armstrong, J. D. and Metcalfe, N. B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proc. R. Soc. B: Biol. Sci.* **278**, 3465-3473. doi.org/10.1098/rspb.2011.1778

Careau, V., Thomas, D., Humphries, M. M. and Réale, D. (2008). Energy metabolism and animal personality. *Oikos* **117**, 641-653. doi.org/10.1111/j.0030-1299.2008.16513.x

Carey, N. and Sigwart, J. D. (2014). Size matters: plasticity in metabolic scaling shows body-size may modulate responses to climate change. *Biol. Lett.* **10**, 20140408. doi.org/10.1098/rsbl.2014.0408

Carey, N., Sigwart, J. D. and Richards, J. G. (2013). Economies of scaling: more evidence that allometry of metabolism is linked to activity, metabolic rate and habitat. *J. Exp. Mar. Biol. Ecol.* **439**, 7-14. doi.org/10.1016/j.jembe.2012.10.013

Carey, N., Harianto, J. and Byrne, M. (2016). Sea urchins in a high-CO_2 world: partitioned effects of body size, ocean warming and acidification on metabolic rate. *J. Exp. Biol.* **219**, 1178-1186. doi: 10.1242/jeb.136101

Carlini, D. B., Manning, J., Sullivan, P. G., & Fong, D. W. (2009). Molecular genetic variation and population structure in morphologically differentiated cave and surface populations of the freshwater amphipod *Gammarus minus*. *Mol. Ecol.* **18**, 1932-1945. doi.org/10.1111/j.1365-294X.2009.04161.x

Chown, S. L., Marais, E., Terblanche, J. S., Klok, C. J., Lighton, J. R. B. and Blackburn, T. M. (2007). Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. *Funct. Ecol.* **21**, 282-290. doi.org/10.1111/j.1365-2435.2007.01245.x

Claireaux, G. and Lagardère, J. P. (1999). Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. *J. Sea Res*. **42**, 157-168. doi.org/10.1016/S1385-1101(99)00019-2

Clarke, A. (2004). Is there a universal temperature dependence of metabolism? *Funct. Ecol.* **18**, 252-256. doi.org/10.1111/j.0269-8463.2004.00842.x

Cooper, C. J., Mueller, C. A. and Eme, J. (2019). Temperature tolerance and oxygen consumption of two South American tetras, *Paracheirodon innessi* and *Hyphessobrycon herbertaxelrodi*. *J. Thermal Biol.* **86**, 102434. doi.org/10.1016/j.jtherbio.2019.102434

Czarnołęski, M., Kozłowski, J., Dumiot, G., Bonnet, J. C., Mallard, J. and Dupont-Nivet, M. (2008). Scaling of metabolism in *Helix aspersa* snails: changes through ontogeny and response to selection for increased size. *J. Exp. Biol.* **211**, 391-400. doi: 10.1242/jeb.013169

Darveau, C. A., Suarez, R. K., Andrews, R. D. and Hochachka, P. W. (2002). Allometric cascade as a unifying principle of body mass effects on metabolism. *Nature* **417**, 166-170. doi.org/10.1038/417166a

da Silva Vianna, B., Miyai, C. A., Augusto, A. and Costa, T. M. (2020). Effects of temperature increase on the physiology and behavior of fiddler crabs. *Physiol. Behav.* **215**, 112765. doi.org/10.1016/j.physbeh.2019.112765

Davison, J. (1955). Body weight, cell surface, and metabolic rate in anuran Amphibia. *Biol. Bull.* **109**, 407–419. doi.org/10.2307/1539173

Dehnel, P. A. (1960). Effect of temperature and salinity on the oxygen consumption of two intertidal crabs. *Biol. Bull*. **118**, 215-249. doi.org/10.2307/1538998

Dell, A. I., Pawar, S. and Savage, V. M. (2011). Systematic variation in the temperature dependence of physiological and ecological traits. *Proc. Natl. Acad. Sci. U.S.A.* 108, 10591-10596. doi.org/10.1073/pnas.1015178108

DeLong, J. P., Gibert, J. P., Luhring, T. M., Bachman, G., Reed, B., Neyer, A. and Montooth, K. L. (2017). The combined effects of reactant kinetics and enzyme stability explain the temperature dependence of metabolic rates. *Ecol. Evol.* **7**, 3940-3950. doi.org/10.1002/ece3.2955

Dick, J. T. (1995). The cannibalistic behaviour of two *Gammarus* species (Crustacea: Amphipoda). *J. Zool.* **236**, 697-706. doi.org/10.1111/j.1469-7998.1995.tb02740.x

Dlugosz, E. M., Chappell, M. A., Meek, T. H., Szafrańska, P. A., Zub, K., Konarzewski, M., Jones, J. H., Bicudo, J. E. P. W., Nespolo, R. F., Careau, V. and Garland, T. (2013). Phylogenetic analysis of mammalian maximal oxygen consumption during exercise. *J. Exp. Biol.* **216**, 4712-4721. doi: 10.1242/jeb.088914

Elliott, J. M. (2002). A continuous study of the total drift of freshwater shrimps, *Gammarus pulex*, in a small stony stream in the English Lake District. *Freshwater Biol.* **47**, 75-86. doi.org/10.1046/j.1365-2427.2002.00782.x

Finn, R. N., Rønnestad, I., van der Meeren, T. and Fyhn, H. J. (2002). Fuel and metabolic scaling during the early life stages of Atlantic cod *Gadus morhua*. *Mar. Ecol. Prog. Ser.* **243**, 217-234. doi:10.3354/meps243217

Foucreau, N., Cottin, D., Piscart, C. and Hervant, F. (2014). Physiological and metabolic responses to rising temperature in *Gammarus pulex* (Crustacea) populations living under continental or Mediterranean climates. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* **168**, 69-75. doi.org/10.1016/j.cbpa.2013.11.006

Fossen, E. I., Pélabon, C. and Einum, S. (2019). Genetic and environmental effects on the scaling of metabolic rate with body size. *J. Exp. Biol.* **222**, jeb193243. doi: 10.1242/jeb.193243

Friberg, N., Andersen, T. H., Hansen, H. O., Iversen, T. M., Jacobsen, D., Krøjgaard, L. and Larsen, S. E. (1994). The effect of brown trout (*Salmo trutta* L.) on stream invertebrate drift, with special reference to *Gammarus pulex* L. *Hydrobiologia* **294**, 105-110. doi.org/10.1007/BF00016850

Galic, N. and Forbes, V. E. (2017). Effects of temperature on the performance of a freshwater amphipod. *Hydrobiologia* **785**, 35-46. doi.org/10.1007/s10750-016-2901-9

Gillooly, J. F., Allen, A. P., Savage, V. M., Charnov, E. L., West, G. B. and Brown, J. H. (2006). Response to Clarke and Fraser: effects of temperature on metabolic rate. *Funct. Ecol.* **20**, 400-404. www.jstor.org/stable/3806577

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. and Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science* **293**, 2248-2251. doi: 10.1126/science.1061967

Gjoni, V., Basset, A. and Glazier, D. S. (2020). Temperature and predator cues interactively affect ontogenetic metabolic scaling of aquatic amphipods. *Biol. Lett.* **16**, 20200267. doi.org/10.1098/rsbl.2020.0267.

Glazier, D. S. (1991). Separating the respiration rates of embryos and brooding females of *Daphnia magna*: implications for the cost of brooding and the allometry of metabolic rate. *Limnol. Oceanogr*. **36**, 354-361. doi.org/10.4319/lo.1991.36.2.0354

Glazier, D. S. (1999). Variation in offspring investment within and among populations of *Gammarus minus* Say (Crustacea: Amphipoda) in ten mid-Appalachian springs (U.S.A.). *Archiv Hydrobiol.* **146**, 257-284. doi:10.1127/archiv-hydrobiol/146/1999/257

Glazier, D. S. (2000). Is fatter fitter? Body storage and reproduction in ten populations of the freshwater amphipod *Gammarus minus*. *Oecologia* **122**, 335-345. doi.org/10.1007/s004420050039

Glazier, D. S. (2005). Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. *Biol. Rev.* **80**, 611-662. doi.org/10.1017/S1464793105006834

Glazier, D. S. (2006). The 3/4-power law is not universal: evolution of isometric, ontogenetic metabolic scaling in pelagic animals. *BioScience* **56**, 325-332. doi.org/10.1641/0006-3568(2006)56[325:TPLINU]2.0.CO;2

Glazier, D. S. (2008). Effects of metabolic level on the body size scaling of metabolic rate in birds and mammals. *Proc. R. Soc. B: Biol. Sci*. **275**, 1405-1410. doi.org/10.1098/rspb.2008.0118

Glazier, D. S. (2009a). Activity affects intraspecific body-size scaling of metabolic rate in ectothermic animals. *J. Comp. Physiol. B* **179**, 821-828. doi.org/10.1007/s00360-009-0363-3

Glazier, D. S. (2009b). Ontogenetic body-mass scaling of resting metabolic rate covaries with species-specific metabolic level and body size in spiders and snakes. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* **153**, 403-407. doi.org/10.1016/j.cbpa.2009.03.020

Glazier, D. S. (2010). A unifying explanation for diverse metabolic scaling in animals and plants. *Biol. Rev.* **85**, 111-138. doi.org/10.1111/j.1469-185X.2009.00095.x

Glazier, D. S. (2013). Log-transformation is useful for examining proportional relationships in allometric scaling. *J. Theor. Biol.* **334**, 200-203. doi: 10.1016/j.jtbi.2013.06.017

Glazier, D. S. (2014a). Amphipoda. In *Reference Module in Earth Systems and Environmental Sciences* (ed. S. Elias). Waltham, MA: Elsevier. doi:10.1016/B978-0-12-409548-9.09437-9

Glazier, D. S. (2014b). Metabolic scaling in complex living systems. *Systems* **2**, 451-540. doi.org/10.3390/systems2040451

Glazier, D. S. (2014c). Scaling of metabolic scaling within physical limits. *Systems* **2**, 425-450. doi.org/10.3390/systems2040425

Glazier, D. S. (2014d). Springs. In *Reference Module in Earth Systems and Environmental Sciences* (ed. S. Elias). Waltham, MA: Elsevier. doi:10.1016/B978-0-12-409548-9.09322-2

Glazier, D. S. (2015). Is metabolic rate a universal 'pacemaker' for biological processes? *Biol. Rev.* **90**, 377-407. doi.org/10.1111/brv.12115

Glazier, D. S. (2018a). Effects of contingency versus constraints on the body-mass scaling of metabolic rate. *Challenges* **9**, 4. doi.org/10.3390/challe9010004

Glazier, D. S. (2018b). Rediscovering and reviving old observations and explanations of metabolic scaling in living systems. *Systems* **6**, 4. doi.org/10.3390/systems6010004

Glazier, D. S. (2018c). Resource supply and demand both affect metabolic scaling: a response to Harrison. *Trends Ecol. Evol.* **33**, 237-238. doi: 10.1016/j.tree.2018.01.006

Glazier, D. S. (2020). Activity alters how temperature influences intraspecific metabolic scaling: testing the metabolic-level boundaries hypothesis. *J. Comp. Physiol. B* **190**, 445-454. doi.org/10.1007/s00360-020-01279-0

Glazier, D. S., Borrelli, J. J. and Hoffman, C. L. (2020). Effects of fish predators on the mass-related energetics of a keystone freshwater crustacean. *Biology* **9**, 40. doi.org/10.3390/biology9030040

Glazier, D. S., Butler, E. M., Lombardi, S. A., Deptola, T. J., Reese, A. J. and Satterthwaite, E. V. (2011). Ecological effects on metabolic scaling: amphipod responses to fish predators in freshwater springs. *Ecol. Monogr.* **81**, 599-618. doi.org/10.1890/11-0264.1

Glazier, D. S. and Deptola, T. J. (2011). The amphipod *Gammarus minus* has larger eyes in freshwater springs with numerous fish predators. *Invert. Biol.* **130**, 60-67. doi.org/10.1111/j.1744-7410.2010.00220.x

Glazier D. S. and Gooch, J. L. (1987). Macroinvertebrate assemblages in Pennsylvania (USA) springs. *Hydrobiologia* **150**, 33-43. doi.org/10.1007/BF00006608

Glazier, D. S., Hirst, A. G. and Atkinson, D. (2015). Shape shifting predicts ontogenetic changes in metabolic scaling in diverse aquatic invertebrates. *Proc. R. Soc. London B: Biol. Sci.* **282**, 20142302. doi.org/10.1098/rspb.2014.2302

Glazier, D. S., Horne, M. T. and Lehman, M. E. (1992). Abundance, body composition and reproductive output of *Gammarus minus* (Crustacea : Amphipoda) in ten cold springs differing in pH and ionic content. *Freshwater Biol.* **28**, 149-163. doi.org/10.1111/j.1365-2427.1992.tb00572.x

Glazier, D. S. and Paul, D. A. (2017). Ecology of ontogenetic body-mass scaling of gill-surface area in a freshwater crustacean. *J. Exp. Biol.* **220**, 2120-2127. doi: 10.1242/jeb.155242

Glazier, D. S. and Sparks, B. L. (1997). Energetics of amphipods in ion-poor waters: stress resistance is not invariably linked to low metabolic rates. *Funct. Ecol.* **11**, 126-128. www.jstor.org/stable/2390555

Glover, D. C., DeVries, D. R. and Wright, R. A. (2012). Effects of temperature, salinity and body size on routine metabolism of coastal largemouth bass *Micropterus salmoides*. *J. Fish Biol.* **81**, 1463-1478. doi.org/10.1111/j.1095-8649.2012.03385.x

Gooch, J. L. (1990). Spatial genetic patterns in relation to regional history and structure: *Gammarus minus* (Amphipoda) in Appalachian watersheds. *Am. Midl. Nat.* **124**, 93–104. doi: 10.2307/2426082

Gooch, J. L. and Glazier, D. S. (1991). Temporal and spatial patterns in mid-Appalachian springs. *Mem. Entomol. Soc. Can.* **155**, 29–49. doi.org/10.4039/entm123155029-1

Gudowska, A., Schramm, B. W., Czarnoleski, M., Antoł, A., Bauchinger, U. and Kozłowski, J. (2017). Mass scaling of metabolic rates in carabid beetles (Carabidae)—the importance of phylogeny, regression models and gas exchange patterns. *J. Exp. Biol.* **220**, 3363-3371. doi: 10.1242/jeb.159293

Handelsman, C. A., Broder, E. D., Dalton, C. M., Ruell, E. W., Myrick, C. A., Reznick, D. N. and Ghalambor, C. K. (2013). Predator-induced phenotypic plasticity in metabolism and rate of growth: rapid adaptation to a novel environment. *Integr. Comp. Biol.* **53**, 975-988. doi.org/10.1093/icb/ict057

Harianto, J., Nguyen, H. D., Holmes, S. P. and Byrne, M. (2018). The effect of warming on mortality, metabolic rate, heat-shock protein response and gonad growth in thermally acclimated sea urchins (*Heliocidaris erythrogramma*). *Mar. Biol.* **165**, 96. doi.org/10.1007/s00227-018-3353-8

Harrison, J. F. (2017). Do performance–safety tradeoffs cause hypometric metabolic scaling in animals? *Trends Ecol. Evol.* **32**, 653–664. doi.org/10.1016/j.tree.2017.05.008

Hatton, I. A., Dobson, A. P., Storch, D., Galbraith, E. D. and Loreau, M. (2019). Linking scaling laws across eukaryotes. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 21616-21622. doi.org/10.1073/pnas.1900492116

Hawlena D. and Schmitz, O. J. (2010). Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *Am. Nat.* **176**, 537–556. doi.org/10.1086/656495

Hemmingsen, A. M. (1960). Energy metabolism as related to body size and respiratory surfaces, and its evolution. *Rep. Steno Mem. Hosp. Nord. Insulin Lab. (Copenhagen)* **9**, 1–110.

Heusner, A. A. (1982). Energy metabolism and body size I. Is the 0.75 mass exponent of Kleiber's equation a statistical artifact? *Resp. Physiol.* **48**, 1-12. doi.org/10.1016/0034-5687(82)90046-9

Hirst, A. G., Glazier, D. S. and Atkinson, D. (2014). Body shape shifting during growth permits tests that distinguish between competing geometric theories of metabolic scaling. *Ecol. Lett.* **17**, 1274-1281. doi.org/10.1111/ele.12334

Hou, C., Zuo, W., Moses, M. E., Woodruff, W. H., Brown, J. H. and West, G. B. (2008). Energy uptake and allocation during ontogeny. *Science* **322**, 736-739. doi: 10.1126/science.1162302

Issartel, J., Hervant, F., Voituron, Y., Renault, D. and Vernon, P. (2005). Behavioural, ventilatory and respiratory responses of epigean and hypogean crustaceans to different temperatures. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* **141**, 1-7. doi.org/10.1016/j.cbpb.2005.02.013

Janssens, L., Van Dievel, M. and Stoks, R. (2015). Warming reinforces nonconsumptive predator effects on prey growth, physiology, and body stoichiometry. *Ecology* **96**, 3270-3280. doi.org/10.1890/15-0030.1

Jenio, F. (1979). Predation on freshwater gammarids (Crustacea: Amphipoda). *Proc. West Virginia Acad. Sci.* **51**, 67-73.

Jermacz, Ł., Kletkiewicz, H., Krzyżyńska, K., Klimiuk, M. and Kobak, J. (2020a). Does global warming intensify cost of antipredator reaction? A case study of freshwater amphipods. *Sci. Total Environ.* **742**, 140474. doi.org/10.1016/j.scitotenv.2020.140474

Jermacz, Ł., Nowakowska, A., Kletkiewicz, H. and Kobak, J. (2020b). Experimental evidence for the adaptive response of aquatic invertebrates to chronic predation risk. *Oecologia* **192**, 341-350. doi.org/10.1007/s00442-020-04594-z

Kane, T. C., Culver, D. C. and Jones, R. T. 1992. Genetic structure of morphologically differentiated populations of the amphipod *Gammarus minus*. *Evolution* **46**, 272-278. doi: 10.2307/2409822

Kearney, M. R. and White, C. R. (2012). Testing metabolic theories. *Am. Nat.* **180**, 546-565. doi.org/10.1086/667860

Kerkhoff, A. J. and Enquist, B. J. (2009). Multiplicative by nature: why logarithmic transformation is necessary in allometry. *J. Theor. Biol.* **257**, 519-521. doi.org/10.1016/j.jtbi.2008.12.026

Killen, S. S., Costa, I., Brown, J. A., & Gamperl, A. K. (2007). Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. *Proc. R. Soc. London B: Biol. Sci.* **274**, 431-438. doi.org/10.1098/rspb.2006.3741

Killen, S. S., Atkinson, D. and Glazier, D. S. (2010). The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecol. Lett.* **13**, 184-193. doi.org/10.1111/j.1461-0248.2009.01415.x

Kleiber, M. (1932). Body size and metabolism. *Hilgardia* **6**, 315–353. doi:10.3733/hilg.v06n11p315

Kleiber, M. (1961). *The Fire of Life*. New York, NY: Wiley.

Kooijman, S. A. L. M. (2000). *Dynamic Energy and Mass Budgets in Biological Systems*. Cambridge: Cambridge University Press.

Konarzewski, M. and Książek, A. (2013). Determinants of intra-specific variation in basal metabolic rate. *J. Comp. Physiol. B*, **183**, 27-41. doi.org/10.1007/s00360-012-0698-z

Kozłowski, J., Konarzewski, M. and Gawelczyk, A. T. (2003). Cell size as a link between noncoding DNA and metabolic rate scaling. *Proc. Natl. Acad. Sci. USA* **100**, 14080–14085. doi.org/10.1073/pnas.2334605100

Kratina, P., Watts, T. J., Green, D. S., Kordas, R. L. and O'Gorman, E. J. (2019). Interactive effects of warming and microplastics on metabolism but not feeding rates of a key freshwater detritivore. *Environ. Pollut.* **255**, 113259. doi.org/10.1016/j.envpol.2019.113259

Kremer, P. (1977). Respiration and excretion by the ctenophore *Mnepiopsis leidyi*. *Mar. Biol.* **44**, 43-50. doi.org/10.1007/BF00386903

Krogh, A. (1916). *The Respiratory Exchange of Animals and Man*. London: Longmans, Green and Co.

Laidler, K. J. (1984). The development of the Arrhenius equation. *J. Chem. Ed*. **61**, 494-498. doi: 10.1021/ed061p494

Larson, R. J. (1987). Respiration and carbon turnover rates of medusae from the NE Pacific. *Comp. Biochem. Physiol. A: Physiol.* **87**, 93-100. doi.org/10.1016/0300-9629(87)90430-0

Lau, J. A. and terHorst, C. P. (2019). Evolutionary responses to global change in species-rich communities. *Ann. N. Y. Acad. Sci.* doi: 10.1111/nyas.14221

Laws, A. N. (2017). Climate change effects on predator–prey interactions. *Curr. Opin. Insect Sci.* **23**, 28-34. doi.org/10.1016/j.cois.2017.06.010

Leiva, F. P., Garcés, C., Verberk, W. C., Care, M., Paschke, K. and Gebauer, P. (2018). Differences in the respiratory response to temperature and hypoxia across four life-stages of the intertidal porcelain crab *Petrolisthes laevigatus*. *Mar. Biol*. **165**, 146. doi.org/10.1007/s00227-018-3406-z

Lewis, S. E., Dick, J. T., Lagerstrom, E. K. and Clarke, H. C. (2010). Avoidance of filial cannibalism in the amphipod *Gammarus pulex*. *Ethology* **116**, 138-146. doi.org/10.1111/j.1439-0310.2009.01726.x

Luhring, T. M., Vavra, J. M., Cressler, C. E. and DeLong, J. P. (2019). Phenotypically plastic responses to predation risk are temperature dependent. *Oecologia* **191**, 709-719. doi.org/10.1007/s00442-019-04523-9

MacNeil, C., Dick, J. T. and Elwood, R. W. (1997). The trophic ecology of freshwater *Gammarus* spp. (Crustacea: Amphipoda): problems and perspectives concerning the functional feeding group concept. *Biol. Rev.* **72**, 349-364. doi.org/10.1017/S0006323196005038

MacNeil, C., Dick, J. T. and Elwood, R. W. (1999). The dynamics of predation on *Gammarus* spp. (Crustacea: Amphipoda). *Biol. Rev.* **74**, 375-395. doi.org/10.1111/j.1469-185X.1999.tb00035.x

Maino, J. L. and Kearney, M. R. (2014). Ontogenetic and interspecific metabolic scaling in insects. *Am. Nat.* **184**, 695-701. doi.org/10.1086/678401

Marchant, R. and Hynes, H. B. N. (1981). Field estimates of feeding rate for *Gammarus pseudolimnaeus* (Crustacea: Amphipoda) in the Credit River, Ontario. *Freshwater Biol.* **11**, 27-36. doi.org/10.1111/j.1365-2427.1981.tb01240.x

Marshall, D. J. and McQuaid, C. D. (2011). Warming reduces metabolic rate in marine snails: adaptation to fluctuating high temperatures challenges the metabolic theory of ecology. *Proc. R. Soc. B: Biol. Sci.* **278**, 281-288. doi.org/10.1098/rspb.2010.1414

Mathot, K. J., Dingemanse, N. J. and Nakagawa, S. (2019). The covariance between metabolic rate and behaviour varies across behaviours and thermal types: meta-analytic insights. *Biol. Rev.* **94**, 1056-1074. doi.org/10.1111/brv.12491

McGrath, K. E., Peeters, E. T., Beijer, J. A. and Scheffer, M. (2007). Habitat-mediated cannibalism and microhabitat restriction in the stream invertebrate *Gammarus pulex*. *Hydrobiologia* **589**, 155-164. doi.org/10.1007/s10750-007-0731-5

Moffett, E. R., Fryxell, D. C., Palkovacs, E. P., Kinnison, M. T. and Simon, K. S. (2018). Local adaptation reduces the metabolic cost of environmental warming. *Ecology* **99**, 2318-2326.

Moran, D. and Wells, R. M. (2007). Ontogenetic scaling of fish metabolism in the mouse-to-elephant mass magnitude range. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* **148**, 611-620. doi.org/10.1016/j.cbpa.2007.08.006

Moses, M. E., Hou, C., Woodruff, W. H., West, G. B., Nekola, J. C., Zuo, W. and Brown, J. H. (2008). Revisiting a model of ontogenetic growth: estimating model parameters from theory and data. *Am. Nat.* **171**, 632-645. doi.org/10.1086/587073

Mosser, M. L. and Hettler, W. F. (1989). Routine metabolism of juvenile spot, *Leiostomus xanthurus* (Lacépède), as a function of temperature, salinity and weight. *J. Fish Biol.* **35**, 703-707. doi.org/10.1111/j.1095-8649.1989.tb03021.x

Newell, R. C., Ahsanullah, M. and Pye, V. I. (1972). Aerial and aquatic respiration in the shore crab *Carcinus maenas* (L.). *Comp. Biochem. Physiol. A: Physiol.* **43**, 239-252. doi.org/10.1016/0300-9629(72)90182-X

Newell, R. C. and Northcroft, H. R. (1967). A reinterpretation of the effects of temperature on the metabolism of certain marine invertebrates. *J. Zool.* **151**, 277–298. doi.org/10.1111/j.1469-7998.1967.tb02878.x

Newell, R. C., Roy, A., & Armitage, K. B. (1976). An analysis of factors affecting the oxygen consumption of the isopod *Ligia oceanica*. *Physiol. Zool.* **49**, 109-137. doi.org/10.1086/physzool.49.1.30155682

Newman, R. M. and Waters, T. F. (1984) Size-selective predation on *Gammarus pseudolimnaeus* by trout and sculpins. *Ecology* **65**, 535–1545. doi.org/10.2307/1939133

Norin, T. and Metcalfe, N. B. (2019). Ecological and evolutionary consequences of metabolic rate plasticity in response to environmental change. *Phil. Trans. Roy. Soc. B* **374**, 20180180. doi.org/10.1098/rstb.2018.0180

Ohlberger, J. Mehner, T., Staaks, G., & Hölker, F. (2012). Intraspecific temperature dependence of the scaling of metabolic rate with body mass in fishes and its ecological implications. *Oikos* **121**, 245–251. doi.org/10.1111/j.1600-0706.2011.19882.x

Paine, R. T. (1971). Energy flow in a natural population of the herbivorous gastropod *Tegula funebralis*. *Limnol. Oceanogr.* **16**, 86-98. doi.org/10.4319/lo.1971.16.1.0086

Parry, G. D. (1983). The influence of the cost of growth on ectotherm metabolism. *J. Theor. Biol.* **101**, 453–477. doi.org/10.1016/0022-5193(83)90150-9

Peeters, E. T. H. M., De Lange, H. J. and Lürling, M. (2009). Variation in the behavior of the amphipod *Gammarus pulex*. *Hum. Ecol. Risk Assess.* **15**, 41-52. doi.org/10.1080/10807030802615055

Penick, D. N., Spotila, J. R., O'Connor, M. P., Steyermark, A. C., George, R. H., Salice, C. J. and Paladino, F. V. (1998). Thermal independence of muscle tissue metabolism in the leatherback turtle, *Dermochelys coriacea*. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* **120**, 399-403. doi.org/10.1016/S1095-6433(98)00024-5

Peng, Y., Niklas, K. J., Reich, P. B. and Sun, S. (2010). Ontogenetic shift in the scaling of dark respiration with whole-plant mass in seven shrub species. *Funct. Ecol.* **24**, 502-512. doi.org/10.1111/j.1365-2435.2009.01667.x

Pequeno, P. A., Baccaro, F. B., Souza, J. L. and Franklin, E. (2017). Ecology shapes metabolic and life history scalings in termites. *Ecol. Ent.* **42**, 115-124. doi.org/10.1111/een.12362

Pettersen, A. K., Marshall, D. J. and White, C. R. (2018). Understanding variation in metabolic rate. *J. Exp. Biol.* **221**, jeb166876. doi: 10.1242/jeb.166876

Phillipson, J. and Watson, J. (1965). Respiratory metabolism of the terrestrial isopod *Oniscus asellus* L. *Oikos* **16**, 78-87. doi: 10.2307/3564867

Réveillon, T., Rota, T., Chauvet, É., Lecerf, A. and Sentis, A. (2019). Repeatable inter-individual variation in the thermal sensitivity of metabolic rate. *Oikos* **128**, 1633-1640. doi.org/10.1111/oik.06392

Riisgård, H. U. (1998). No foundation of a '3/4 power scaling law' for respiration in biology. *Ecol. Lett*. **1**, 71-73. doi.org/10.1046/j.1461-0248.1998.00020.x

Sargent, H. (2019). The effect of elevation and temperature on metabolic intensity of *Eulamprus kosciuskoi* in Kosciuszko National Park. *Field Stud. Ecol.* **2**, 1. http://studentjournals.anu.edu.au/index.php/fse/article/view/217

Savage, V. M., Gillooly, J. F., Woodruff, W. H., West, G. B., Allen, A. P., Enquist, B. J. and Brown, J. H. (2004). The predominance of quarter-power scaling in biology. *Funct. Ecol.* **18**, 257-282. doi.org/10.1111/j.0269-8463.2004.00856.x

Scheffler, M. L., Barreto, F. S. and Mueller, C. A. (2019). Rapid metabolic compensation in response to temperature change in the intertidal copepod, *Tigriopus californicus*. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* **230**, 131-137. doi.org/10.1016/j.cbpa.2019.01.017

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J. Exp. Biol*. **218**, 1856-1866. doi: 10.1242/jeb.118851

Schwieterman, G. D., Crear, D. P., Anderson, B. N., Lavoie, D. R., Sulikowski, J. A., Bushnell, P. G. and Brill, R. W. (2019). Combined effects of acute temperature change and elevated pCO_2 on the metabolic rates and hypoxia tolerances of Clearnose Skate (*Rostaraja eglanteria*), Summer Flounder (*Paralichthys dentatus*), and Thorny Skate (*Amblyraja radiata*). *Biology* **8**, 56. doi.org/10.3390/biology8030056

Seibel, B. A., Dymowska, A. and Rosenthal, J. (2007). Metabolic temperature compensation and coevolution of locomotory performance in pteropod molluscs. *Integr. Comp. Biol.* **47**, 880–891. doi.org/10.1093/icb/icm089

Semsar-kazerouni, M. and Verberk, W. C. (2018). It's about time: Linkages between heat tolerance, thermal acclimation and metabolic rate at different temporal scales in the freshwater amphipod *Gammarus fossarum* Koch, 1836. *J. Therm. Biol.* **75**, 31-37. doi.org/10.1016/j.jtherbio.2018.04.016

Shokri, M., Ciotti, M., Vignes, F., Gjoni, V. and Basset, A. (2019). Components of standard metabolic rate variability in three species of gammarids. *Web Ecol.* **19**, 1. doi:10.5194/we-19-1-2019

Sibly, R. M., Brown, J. H. and Kodric-Brown, A., editors. (2012). *Metabolic Ecology: a Scaling Approach*. Chichester, UK: Wiley-Blackwell.

Smith, R. J. (2009). Use and misuse of the reduced major axis for line-fitting. *Am. J Phys. Anthropol.* **140**, 476–486. doi.org/10.1002/ajpa.21090

Smith, R. W. (1997). Visual hypothesis testing with confidence intervals. In *Proceedings of the Twenty-Second Annual SAS Users Group International Conference*, pp. 1252-1257. Cary, NC: SAS Institute.

Spanopoulos-Hernández, M., Martínez-Palacios, C. A., Vanegas-Pérez, R. C., Rosas, C. and Ross, L. G. (2005). The combined effects of salinity and temperature on the oxygen consumption of juvenile shrimps *Litopenaeus stylirostris* (Stimpson, 1874). *Aquaculture* **244**, 341-348. doi.org/10.1016/j.aquaculture.2004.11.023

Speakman, J. R., Król, E. and Johnson, M. S. (2004). The functional significance of individual variation in basal metabolic rate. *Physiol. Biochem. Zool.* **77**, 900-915. doi.org/10.1086/427059

Stubbington, R., Hogan, J. P. and Wood, P. J. (2017). Characterization of the density and body size of a *Gammarus pulex* (Crustacea: Amphipoda) population in subsurface sediments reflects the sampling technique used. *Hydrobiologia* **788**, 293-303. doi.org/10.1007/s10750-016-3008-z

Suarez, R. K. and Darveau, C. A. (2005). Multi-level regulation and metabolic scaling. *J. Exp. Biol.* **208**, 1627-1634.

Sutcliffe, D. W. (1984). Quantitative aspects of oxygen uptake by *Gammarus* (Crustacea, Amphipoda): a critical review. *Freshwater Biol.* **14**, 443-489. doi.org/10.1111/j.1365-2427.1984.tb00168.x

Tan, H., Hirst, A. G., Glazier, D. S. and Atkinson, D. (2019). Ecological pressures and the contrasting scaling of metabolism and body shape in coexisting taxa: cephalopods versus teleost fish. *Phil. Trans. R. Soc. B* **374**, 20180543. doi.org/10.1098/rstb.2018.0543

Terblanche, J. S., Janion, C. and Chown, S. L. (2007). Variation in scorpion metabolic rate and rate–temperature relationships: implications for the fundamental equation of the metabolic theory of ecology. *J. Evol. Biol.* **20**, 1602-1612. doi.org/10.1111/j.1420-9101.2007.01322.x

Thyrring, J., Rysgaard, S., Blicher, M. E. and Sejr, M. K. (2015). Metabolic cold adaptation and aerobic performance of blue mussels (*Mytilus edulis*) along a temperature gradient into the High Arctic region. *Mar. Biol.* **162**, 235-243. doi.org/10.1007/s00227-014-2575-7

Truong, K. N., Vu, N. A., Doan, N. X., Le, M. H., Vu, M. T. and Dinh, K. V. (2020). Predator cues increase negative effects of a simulated marine heatwave on tropical zooplankton. *J. Exp. Mar. Biol. Ecol.* **530**, 151415. doi.org/10.1016/j.jembe.2020.151415

Tseng, M., Bernhardt, J. R. and Chila, A. E. (2019). Species interactions mediate thermal evolution. *Evol. Appl.* **12**, 1463-1474. doi.org/10.1111/eva.12805

Tseng, M. and O'Connor, M. I. (2015). Predators modify the evolutionary response of prey to temperature change. *Biol. Lett.* **11**, 20150798. doi.org/10.1098/rsbl.2015.0798

Vaca, H. F. and White, C. R. (2010). Environmental modulation of metabolic allometry in ornate rainbowfish *Rhadinocentrus ornatus*. *Biol. Lett*. **6**, 136–138. doi.org/10.1098/rsbl.2009.0610

Vernberg, F. J. (1959). Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus *Uca*. II. Oxygen consumption of whole organisms. *Biol. Bull.* **117**, 163-184. doi.org/10.2307/1539048

Verberk, W. C. and Atkinson, D. (2013). Why polar gigantism and Palaeozoic gigantism are not equivalent: effects of oxygen and temperature on the body size of ectotherms. *Funct. Ecol.* **27**, 1275-1285. doi.org/10.1111/1365-2435.12152

Watson, S.-A., Morley, S. A., Bates, A. E., Clark, M. S., Day, R. W., Lamare, M., Martin, S. M., Southgate, P. C., Tan, K. S., Tyler, P. A. and Peck, L. S. (2014). Low global sensitivity of metabolic rate to temperature in calcified marine invertebrates. *Oecologia* **174**, 45-54. doi: 10.1007/s00442-013-2767-8

Weibel, E. R., Bacigalupe, L. D., Schmitt, B. and Hoppeler, H. (2004). Allometric scaling of maximal metabolic rate in mammals: muscle aerobic capacity as determinant factor. *Resp. Physiol. Neurobiol.* **140**, 115-132. doi.org/10.1016/j.resp.2004.01.006

Wesemeier, H. (1960). Untersuchungen über die Stoffwechselreduktion. Ein intra- und interspezifischer vergleich an 17 Molluskenarten. *Z. Vergl. Physiol.* **43**, 1-28. doi.org/10.1007/BF00351199

Wellborn, G. A. (1994). The mechanistic basis of body size differences between two *Hyalella* (Amphipoda) species. *J. Freshwater Ecol*. **9**, 159-168. doi.org/10.1080/02705060.1994.9664443

West, G. B., Brown, J. H. and Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science* **276**, 122-126. doi: 10.1126/science.276.5309.122

West, G. B., Brown, J. H. and Enquist, B. J. (2001). A general model for ontogenetic growth. *Nature* **413**, 628-631. doi.org/10.1038/35098076

White, C. R. (2011). Allometric estimation of metabolic rates in animals. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* **158**, 346–357. doi:10.1016/j.cbpa.2010.10.004

White, C. R., Cassey, P. and Blackburn, T. M. (2007). Allometric exponents do not support a universal metabolic allometry. *Ecology* **88**, 315-323. doi.org/10.1890/05-1883

White, C. R. and Kearney, M. R. (2014). Metabolic scaling in animals: methods, empirical results, and theoretical explanations. *Compr. Physiol.* **4**, 231-256. doi.org/10.1002/cphy.c110049

White, C. R., Kearney, M. R., Matthews, P. G., Kooijman, S. A. and Marshall, D. J. (2011). A manipulative test of competing theories for metabolic scaling. *Am. Nat.* **178**, 746-754. doi.org/10.1086/662666

Wieser, W. (1984). A distinction must be made between the ontogeny and the phylogeny of metabolism in order to understand the mass exponent of energy metabolism. *Resp. Physiol.* **55**, 1-9. doi.org/10.1016/0034-5687(84)90112-9

Wieser, W. (1994). Cost of growth in cells and organisms: general rules and comparative aspects. *Biol. Rev.* **69**, 1–34. doi.org/10.1111/j.1469-185X.1994.tb01484.x

Xie, X. and R. Sun. (1990). The bioenergetics of the southern catfish (*Silurus meridionalis* Chen). I. Resting metabolic rate as a function of body weight and temperature. *Physiol. Zool.* **63**, 1181–1195. doi.org/10.1086/physzool.63.6.30152639
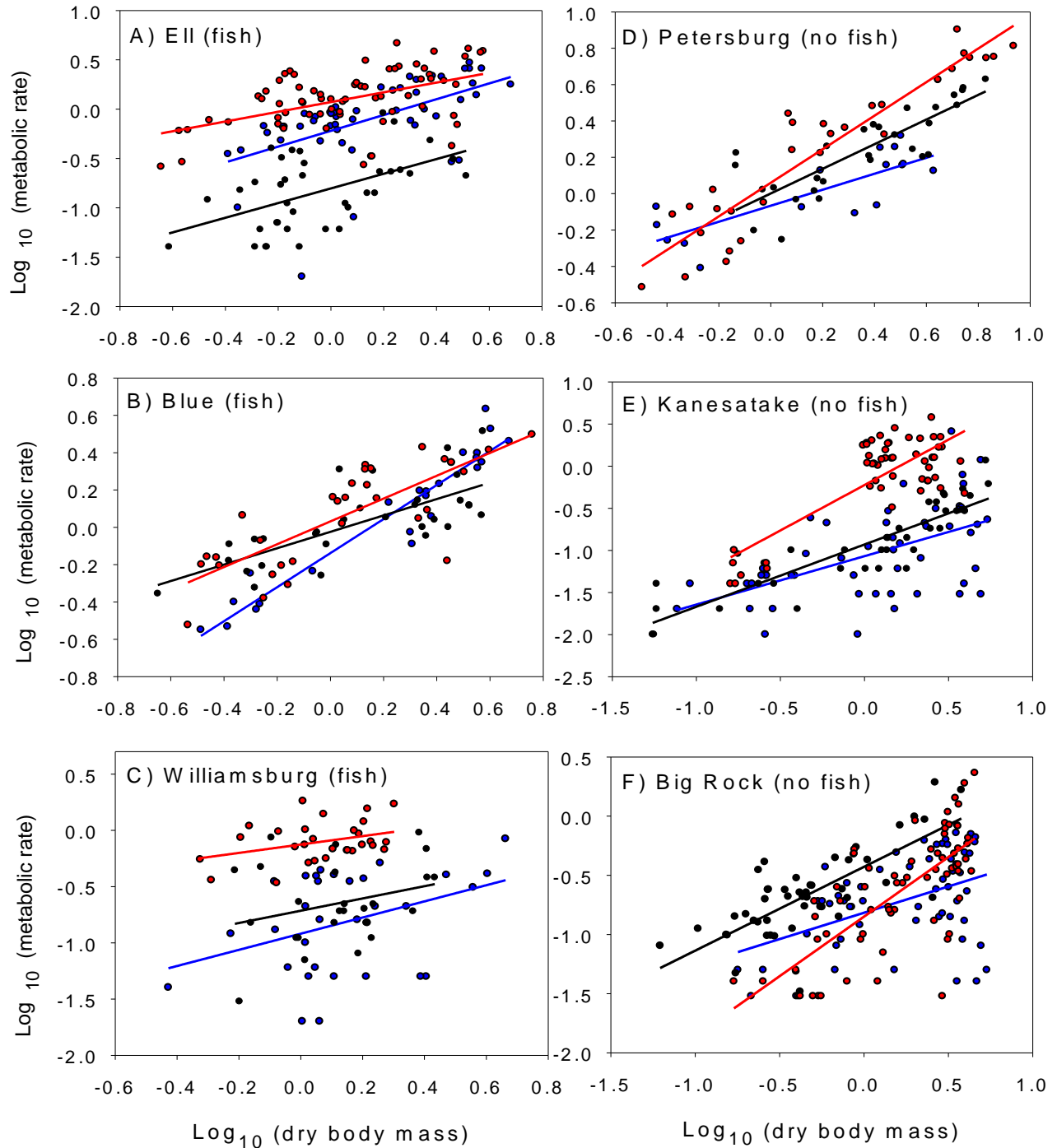
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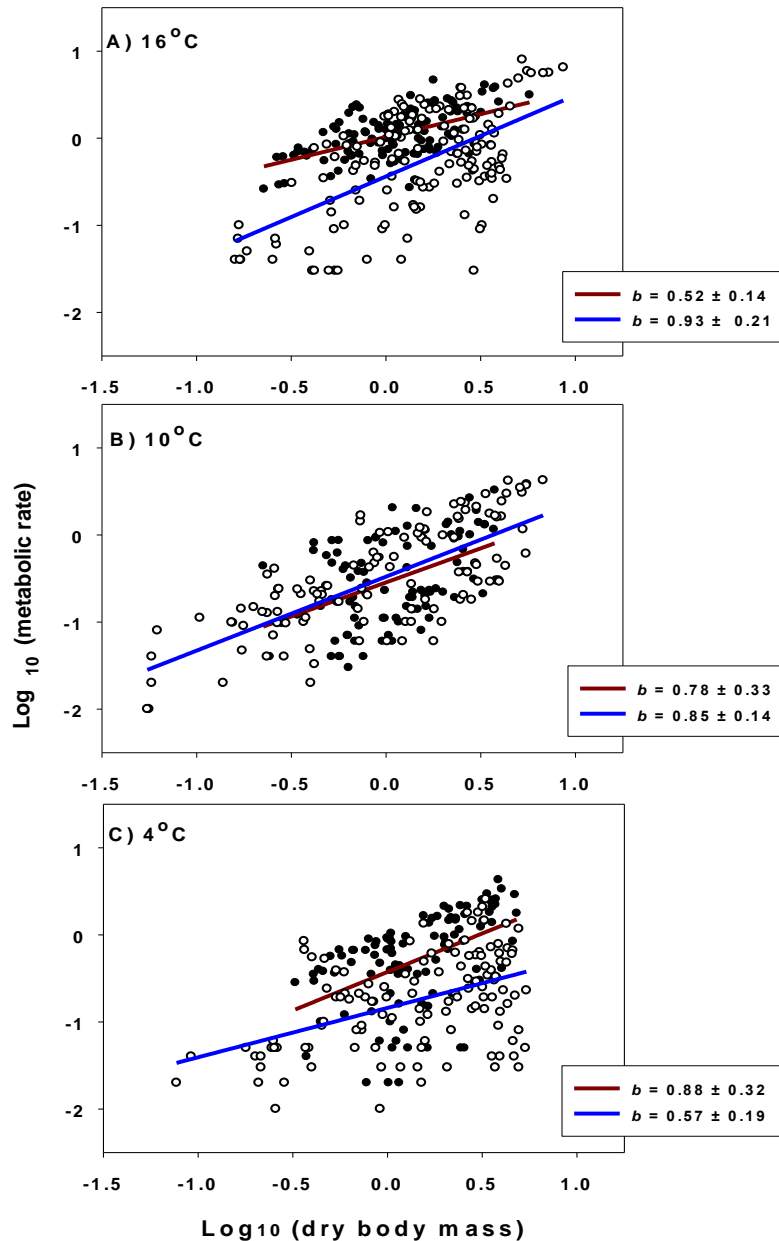
Fig. 1. Resting metabolic rate (μLO$_2$/h) in relation to dry body mass (mg), both log$_{10}$-transformed, for aggregate amphipod (*Gammarus minus*) samples from three lotic springs with fish predators (*Cottus cognatus*), and three without fish predators in six populations of *Gammarus minus*, compared at each of three temperatures. Samples from springs with fish predators: solid points and red regression line; samples from springs without fish predators: open points and blue regression line. Scaling slope (*b*) shown for each regression line. Table 1 gives further statistical details.
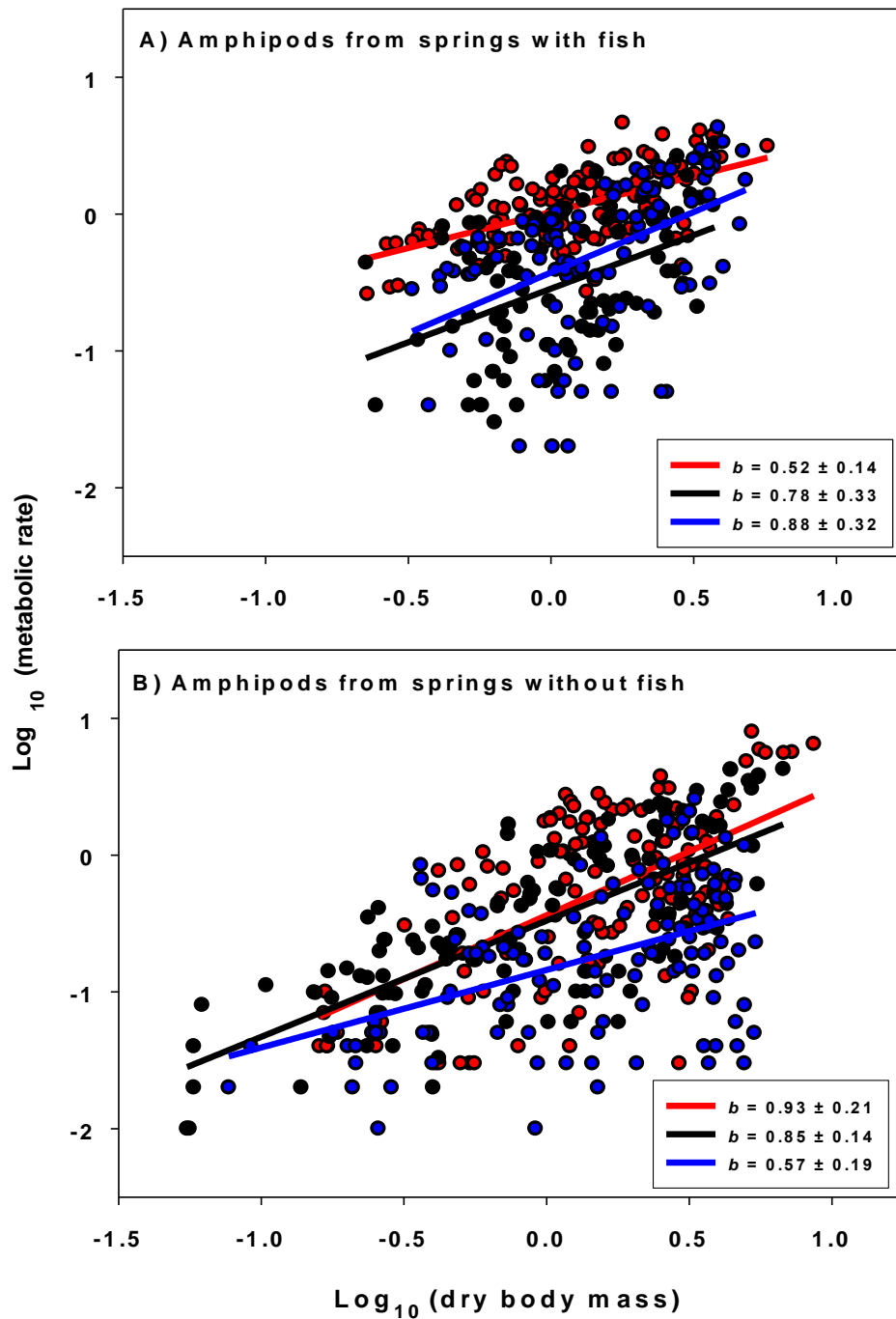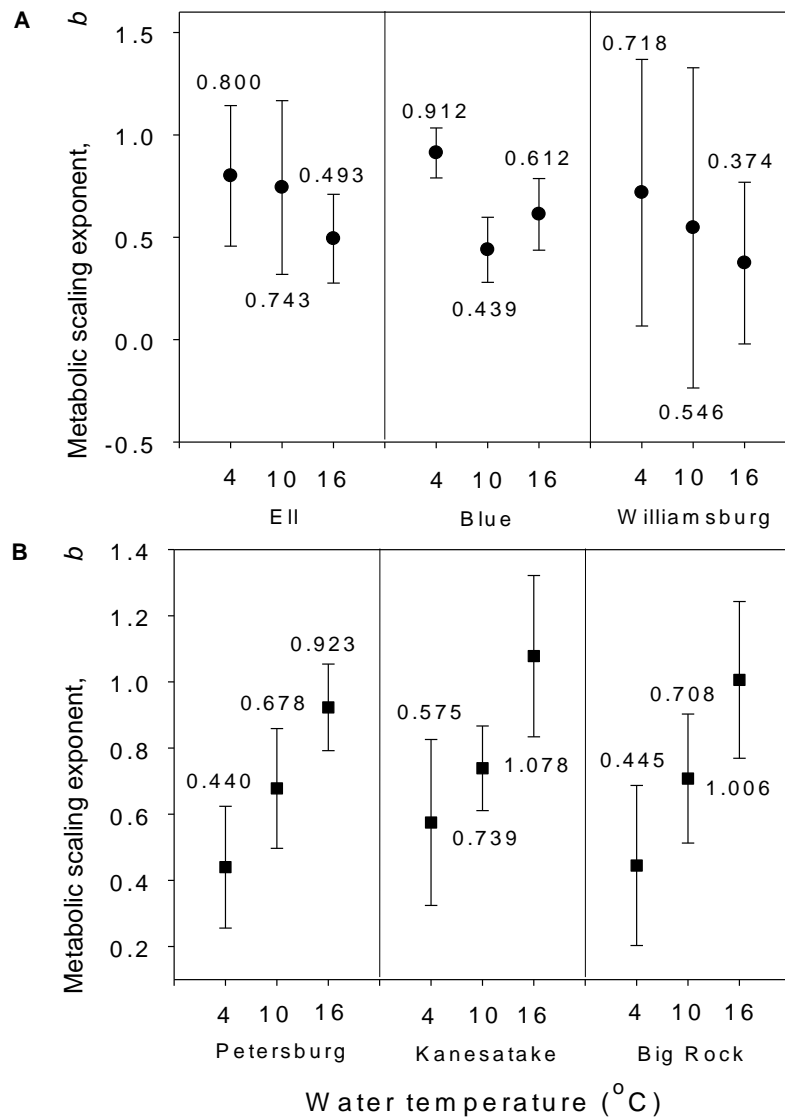
Fig. 2. Resting metabolic rate ($\mu LO_2/h$) in relation to dry body mass (mg), both $\log_{10}$-transformed, for aggregate amphipod (*Gammarus minus*) samples from three lotic springs with fish predators (*Cottus cognatus*), and three without fish predators, each estimated at three temperatures (4, 10 and 16°C: blue, black and red lines and points, respectively). Scaling slope (*b*) shown for each regression line. Table 1 gives further statistical details.

Fig. 3. Resting metabolic rate (µLO₂/h) in relation to dry body mass (mg), both log₁₀-transformed, in six populations of *Gammarus minus* from springs with and without the fish predator *Cottus cognatus*, and at three temperatures (4, 10 and 16°C: blue, black and red lines and points, respectively). Table 3 gives statistical details for regressions lines.

Fig. 4. Scaling slopes ($b \pm 95\%$ C.I.) of resting metabolic rate in relation to dry body mass in six population samples of the amphipod *Gammarus minus* at three water temperatures. Note that the scaling exponents tend to decline with increasing temperature in the populations from springs with fish (A), whereas they tend to increase in the populations from springs without fish (B). As a result, at 4°C, b is always higher in the populations from springs with versus without fish ($t = 4.52$; $P = 0.011$), whereas the opposite occurs at 16°C ($t = 6.21$; $P = 0.0034$). Furthermore, based on simple probability theory, it is statistically significant ($P = 0.0047$) that the scaling exponent is always lowest at 4° C and highest at 16° C in all three of the populations from springs without fish (B). It is also statistically significant ($P = 0.0093$) that the scaling exponent is highest at 4° C in all three of the populations from springs with fish, and lowest at 16°C in two of these populations (A). The probability that both opposite trends would occur simultaneously is only 0.000044 (i.e., 0.0047 x 0.0093), a highly significant pattern.
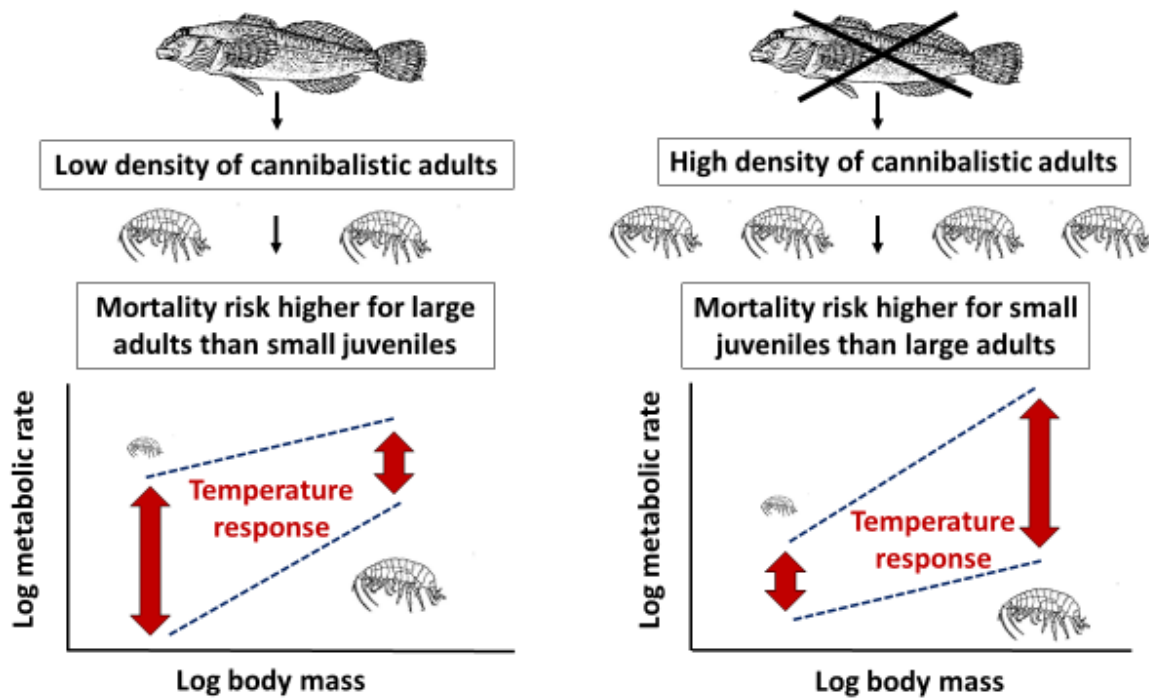
Fig. 5. A schematic of a hypothetical explanation for effects of visually hunting, size-selective fish predators (*Cottus cognatus*) on the size-dependent temperature response of metabolic rate in freshwater amphipods (*Gammarus minus*). According to this hypothesis, higher mortality risk favors reduced responses of activity, growth and supporting metabolism to changes in temperature. Therefore, the body-mass scaling slope for metabolic rate (dashed blue lines) should decrease as temperature increases, for populations exposed to fish predators that cause relatively high adult mortality. However, the metabolic scaling slope should increase as temperature increases, for populations with high densities of cannibalistic adults (made possible by absent fish predators) that cause relatively high juvenile mortality.

Tables

Table 1. Statistical details for scaling relationships between $\log_{10}$ resting metabolic rate ($\mu LO_2/h$) and $\log_{10}$ dry body mass at three temperatures, for amphipod (*Gammarus minus*) samples from three lotic springs with fish predators (*Cottus cognatus*), and three without fish predators in central Pennsylvania, USA.

Sample	T	Slope (± 95% C.I.)	Intercept (± 95% C.I.)	*r*	*n*	*P*
Fish	4	0.884 (±0.317)	-0.427 (±0.105)	0.478	105	<0.00001
Fish	10	0.783 (±0.328)	-0.545 (±0.094)	0.447	92	0.00001
Fish	16	0.524 (±0.144)	0.015 (±0.042)	0.541	127	<0.00001
No fish	4	0.567 (±0.194)	-0.839 (±0.092)	0.465	123	<0.00001
No fish	10	0.850 (±0.143)	-0.479 (±0.076)	0.740	116	<0.00001
No fish	16	0.932 (±0.209)	0.032 (±0.086)	0.591	147	<0.00001

T: water temperature (°C); slopes and intercepts from least squares linear regressions; C.I.: confidence limits; *r*: Pearson's product-moment correlation coefficient; *n*: sample size: *P*: probability that correlation is due to chance.

Table 2. Variation in resting metabolic rate among three temperatures within each of two aggregate samples of the amphipod *Gammarus minus* from lotic springs with fish predators and those without in central Pennsylvania, USA.

Sample	Temperature effect			Body-mass effect			Temperature x body-mass effect		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Fish	2, 318	61.03	<0.00001	1, 318	91.79	<0.00001	2, 318	2.19	0.113
No fish	2, 380	25.59	<0.00001	1, 380	214.86	<0.00001	2, 380	9.07	0.018

Variation was analyzed with ANCOVA (the covariate was dry body mass) using $\log_{10}$-transformed data. df: degrees of freedom; *F*: F statistic; *P*: probability effects were due to chance.

Table 3. Statistical details for scaling relationships between $\log_{10}$ resting metabolic rate ($\mu LO_2/h$) and $\log_{10}$ dry body mass at three temperatures for each of six population samples of the amphipod *Gammarus minus* from lotic springs in central Pennsylvania, USA.

Sample	T	Slope (± 95% C.I.)	Intercept (± 95% C.I.)	r	n	P
Ell (F)	4	0.800 (±0.343)	-0.219 (±0.108)	0.561	50	0.00002
Ell (F)	10	0.743 (±0.424)	-0.803 (±0.109)	0.516	37	0.0011
Ell (F)	16	0.493 (±0.217)	0.072 (±0.066)	0.495	66	0.00002
Blue (F)	4	0.912 (±0.122)	-0.138 (±0.053)	0.959	23	<0.00001
Blue (F)	10	0.439 (±0.159)	-0.024 (±0.057)	0.732	30	<0.00001
Blue (F)	16	0.612 (±0.175)	0.032 (±0.061)	0.794	32	<0.00001
Williamsburg (F)	4	0.718 (±0.651)	-0.919 (±0.177)	0.380	32	0.032
Williamsburg (F)	10	0.546 (±0.424)	-0.714 (±0.109)	0.288	25	0.16
Williamsburg (F)	16	0.374 (±0.217)	-0.126 (±0.070)	0.350	29	0.063
Petersburg (NF)	4	0.440 (±0.184)	-0.066 (±0.077)	0.808	16	0.00015
Petersburg (NF)	10	0.678 (±0.181)	0.001 (±0.084)	0.823	30	<0.00001
Petersburg (NF)	16	0.923 (±0.131)	0.060 (±0.177)	0.939	30	<0.00001
Kanesatake (NF)	4	0.575 (±0.251)	-1.069 (±0.127)	0.549	51	0.00003
Kanesatake (NF)	10	0.739 (±0.128)	-0.930 (±0.077)	0.890	38	<0.00001
Kanesatake (NF)	16	1.078 (±0.244)	-0.225 (±0.093)	0.789	50	<0.00001
Big Rock (NF)	4	0.445 (±0.242)	-0.817 (±0.110)	0.449	56	0.00053
Big Rock (NF)	10	0.708 (±0.194)	-0.428 (±0.099)	0.732	48	<0.00001
Big Rock (NF)	16	1.006 (±0.237)	-0.850 (±0.061)	0.725	67	<0.00001

T: water temperature (^{o}C); slopes and intercepts from least squares linear regressions; C.I.: confidence limits; *r*: Pearson's product-moment correlation coefficient; *n*: sample size: *P*: probability that correlation is due to chance; F: population samples from springs with fish predators (*Cottus cognatus*); NF: population samples from springs without fish predators.

Table 4. Variation in resting metabolic rate among three temperatures within each of six samples of the amphipod *Gammarus minus* from lotic springs in central Pennsylvania, USA.

Sample	Temperature effect			Body-mass effect			Temperature x body-mass effect		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Ell (F)	2, 147	96.24	<0.00001	1, 147	53.55	<0.00001	2, 147	1.38	0.26
Blue (F)	2, 79	7.59	0.00096	1, 79	213.83	<0.00001	2, 79	9.07	0.00029
Williamsburg (F)	2, 80	35.88	<0.00001	1, 80	8.03	0.0058	2, 80	0.295	0.75
Petersburg (NF)	2, 70	3.55	0.034	1, 70	206.23	<0.00001	2, 70	10.13	0.00014
Kanesatake (NF)	2, 133	76.28	<0.00001	1, 133	153.83	<0.00001	2, 133	4.50	0.013
Big Rock (NF)	2, 165	16.35	<0.00001	1, 165	115.95	<0.00001	2, 165	6.36	0.0022

Variation was analyzed with ANCOVA (the covariate was dry body mass) using $\log_{10}$-transformed data. df: degrees of freedom; *F*: F statistic; *P*: probability effects were due to chance. F: populations from springs with fish predators (*Cottus cognatus*); NF: populations from springs without fish predators.

Table S1: Metabolic rate (mL oxygen consumed per hour) and dry body mass (mg) of 710 individual amphipods (*Gammarus minus*) collected from six freshwater spring populations exposed to three different temperatures in the laboratory.

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