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Size matters: plasticity in metabolic scaling shows body-size may modulate responses to climate change

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Variability in metabolic scaling in animals, the relationship between metabolic rate (R) and body mass (M), has been a source of debate and controversy for decades. R is proportional to M^b, the precise value of b much debated, but historically considered equal in all organisms. Recent metabolic theory, however, predicts b to vary among species with ecology and metabolic level, and may also vary within species under different abiotic conditions. Under climate change, most species will experience increased temperatures, and marine organisms will experience the additional stressor of decreased seawater pH ('ocean acidification'). Responses to these environmental changes are modulated by myriad species-specific factors. Body-size is a fundamental biological parameter, but its modulating role is relatively unexplored. Here, we show that changes to metabolic scaling reveal asymmetric responses to stressors across body-size ranges; b is systematically decreased under increasing temperature in three grazing molluscs, indicating smaller individuals were more responsive to warming. Larger individuals were, however, more responsive to reduced seawater pH in low temperatures. These alterations to the allometry of metabolism highlight abiotic control of metabolic scaling, and indicate that responses to climate warming and ocean acidification may be modulated by body-size.

1. Introduction

Effects of environmental change on terrestrial and aquatic species are frequently adverse, but the ultimate intensity of impacts may be modulated by numerous extrinsic factors [1,2], and also body-size [3–5]. Body-size is the major determinant of metabolism [6,7], and metabolic rates of component species ultimately control many aspects of ecosystem functioning [8]. Typically, metabolic rate (R) scales sub-isometrically according to the power law $R = aM^b$, where M is body mass, a the mass coefficient and b a scaling exponent [7,9]. Using log-transformed data, b is the slope of the linear regression $\log R = \log a + \log M \times b$. Historical debate has centred on specific values for b [7,9], commonly proposed 'universal' values including $\frac{2}{3}$ and $\frac{3}{4}$ [6,7,9]. One recent empirically supported model, however, the Metabolic-Level Boundaries (MLB) hypothesis [7], predicts that b should vary between $\frac{2}{3}$ and 1 as a function of species' metabolic level and ecology. Furthermore, this model predicts variation of b within species under different temperatures, through decreasing under increasing temperature [10]. This is because surface-area constraints associated with larger body-sizes become more important as rising temperatures increase the energetic cost of routine metabolism in ectotherms [10]. Another recent model also predicts a negative temperature dependency in metabolic scaling in aquatic organisms, owing to the greater sensitivity of smaller individuals to the increased viscosity of water at colder temperatures, which could increase

the effort, and hence metabolic costs, of moving water around respiratory boundary layers [11].

Systematic variability of b has an important implication: asymmetric changes to metabolism across body-size ranges. If the slope (b) of the relationship between R and M changes, alterations to R may therefore be relatively greater towards one end of the body-size spectrum. Examining changes to metabolic scaling within species therefore provides a test both of the MLB prediction of b decreasing with temperature, and, with the additional stressor of reduced seawater pH, of how body-size may modulate responses to the stressors associated with climate change.

2. Material and methods

Chitons (Mollusca, Polyplacophora) are benthic marine grazers found worldwide, from deep-sea to intertidal habitats. The northeast Pacific fauna represents a rapid radiation in coastal species [12], uniquely displaying four-order magnitude mass variation within a single family, Mopaliidae (2 kg *Cryptochiton stelleri* to 0.1 g *Cyanoplax dentiens*). Despite superficial similarities in morphology and ecology, these species demonstrate substantial differences in metabolic level and scaling [13].

We examined oxygen uptake (VO_2) in three chiton species ($n = 533$) with body-size ranges covering three orders of magnitude (electronic supplementary material, table S1), maintained at two pCO_2 levels and three temperatures (electronic supplementary material, table S2). *Katharina tunicata* (ash-free dry mass (AFDM) 0.004–8.830 g), *Mopalia muscosa* (0.002–4.919 g) and *Tonicella lineata* (0.002–1.279 g) were collected from intertidal sites and held at Friday Harbor Labs, University of Washington. Groups of approximately 35 representing total species size range were randomly allocated among six treatments (control pCO_2: approx. 400 μatm, pH 8.0; elevated pCO_2: approx. 1500 μatm, pH 7.5; at 9°C, 13°C, 17°C). Specimens were distributed among eight replicate tanks within each treatment and not fed during the one-week maintenance period. This maintenance period represented standardization of specimen physiology to stable and presently experienced temperature and pH conditions. Temperatures were well within the natural local range experienced during tidal emersion. Intertidal organisms also frequently experience wide pH gradients (*ca* 6.9–10.1) due to diel interactions between respiration and photosynthesis [14].

Seawater carbonate chemistry (electronic supplementary material, table S2) was maintained by adding CO_2 to an aeration stream to maintain pH setpoints calculated from target pCO_2 levels. Temperature and pH were recorded continuously (Honeywell Durafet III pH electrode; UDA 2182 controller), salinity recorded daily. Detailed chemistry was measured weekly: total alkalinity (A_T) determined using open cell titration; total dissolved inorganic carbon (C_T) monitored by acidification and non-dispersive infra-red (NDIR) quantification of CO_2. Based on these, pCO_2, pH_T (total scale) and saturation states were calculated using CO2calc (electronic supplementary material, table S2).

Respiratory rate (VO_2) (mg O_2 h^{-1}) was determined for each specimen following established protocols [13]. Specimens were sealed in Perspex chambers fitted with stirbars, and oxygen concentrations recorded at 1 s intervals (NeoFox system, Ocean Optics). Control chambers containing only seawater were used to quantify background microbial oxygen consumption. Each specimen was examined only once, and the initial 5% decrease in O_2 discarded from recordings to ensure the specimen was accustomed to the apparatus. No specimens showed signs of stress during experiments, but some aberrant recordings (approx. 20 in total) were discarded from analysis. Specimens were dried at 60°C for 24 h and weighed, incinerated in a muffle furnace at 500°C for 2 h and reweighed, giving AFDM (gram).

Routine oxygen consumption (VO_2) and AFDM were log-transformed and linear ordinary least squares (OLS) regression analysis performed. Linear models were analysed for differences in slope (b) and elevation (i.e. metabolic level) using ANCOVA, with mass, species identity, temperature and pCO_2 as covariates. All data met the homogeneity of variance and normality assumptions of ANCOVA (Bartlett and Shapiro-Wilk, respectively). L_m, the mass-specific metabolic rate (MO_2) predicted by regression models at the midpoint of the species' mass range in log-space, was calculated for each treatment group [10]. Here, differences between regression slopes have a minimal effect on predicted elevation along the y-axis, allowing for comparisons of metabolic level among treatment groups at a standard mass (figure 1).

3. Results and discussion

Two metrics considered together give a comprehensive indication of total-group response to treatment conditions: metabolic rate at log-median mass (L_m), indicating general metabolic level [10]; and the scaling exponent b showing the allometry of metabolism over increasing body-size within a treatment group (figure 1). VO_2 varied significantly with: temperature ($F_{2,497} = 264.97$, $p < 0.001$), pCO_2 level ($F_{1,497} = 9.53$, $p = 0.002$) and species ($F_{2,497} = 34.56$, $p < 0.001$).

Metabolic scaling showed significant differences among species and among the physical treatment parameters. Across our six treatments, in all three species the metabolic scaling exponent (b) (figure 1; electronic supplementary material, table S1) lay entirely within $\frac{2}{3}$ and 1, the boundaries suggested by several models between which b may naturally vary [7,9]. In control pCO_2, b values showed a significant decrease with increasing temperature in all species (two-way interaction of temperature and mass under control pCO_2, $F_{2,248} = 10.855$, $p < 0.001$; figure 1, grey bars and points). This was a consistent pattern, indicated by the non-significant three-way interaction term ($F_{4,248} = 0.53$, $p = 0.72$) when species identity was included as a covariate. This is empirical support for predictions that b is negatively correlated with temperature [7,10,11]. Increased temperature caused an expected increase in metabolism, but this was asymmetric; metabolism in smaller individuals rose relatively more than in larger individuals, reflected in decreasing slope (b) values (e.g. figure 2a).

Unlike temperature, no consistent response of species' metabolism to elevated seawater pCO_2 has been established [1,14]. Current metabolic scaling theory makes no prediction of the response of b to altered pH or pCO_2, although pH sensitivity is hypothesized to be size-dependent in fishes [15]. Scaling exponent values here were largely not different across elevated and control pCO_2 treatments in corresponding temperatures (figure 1). In elevated pCO_2, responses by two species apparently confounded the expected decrease in b with temperature ($F_{2,249} = 0.52$, $p = 0.60$; figure 1, white bars and points). In one group, b in elevated pCO_2 was significantly higher than in control pCO_2 (*K. tunicata*, high temperature: $F_{1,54} = 6.24$, $p = 0.02$, figure 1c). In low temperatures under elevated pCO_2, b was significantly lower in two species (*K. tunicata*: $F_{1,61} = 16.07$, $p < 0.001$; *M. muscosa*: $F_{1,53} = 4.28$, $p = 0.04$). Metabolic depression was also observed in these cold, acidified treatments; VO_2 was
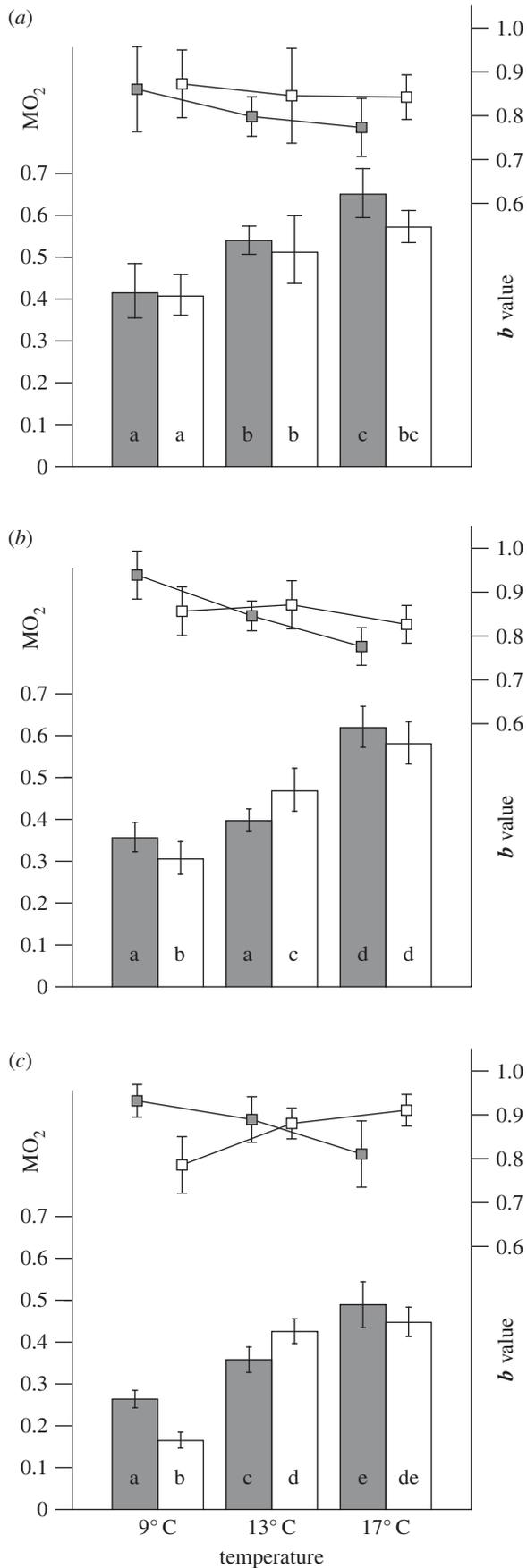
Figure 1. (*a*) *Tonicella lineata*, (*b*) *M. muscosa*, (*c*) *K. tunicata*. For each; treatment group metabolic level, represented as L_m, the mass-specific uptake (MO$_2$; mg O$_2$ h^{-1} g^{-1}) at the log-midpoint of the species mass range (columns, left vertical axis); and scaling exponent b (slope of the linear regression model; points, right vertical axis); both $\pm$ 95% CI. Grey indicates control pCO$_2$; unfilled indicates elevated pCO$_2$. Letter codes within bars denote statistically equivalent metabolic level based on ANCOVA results.
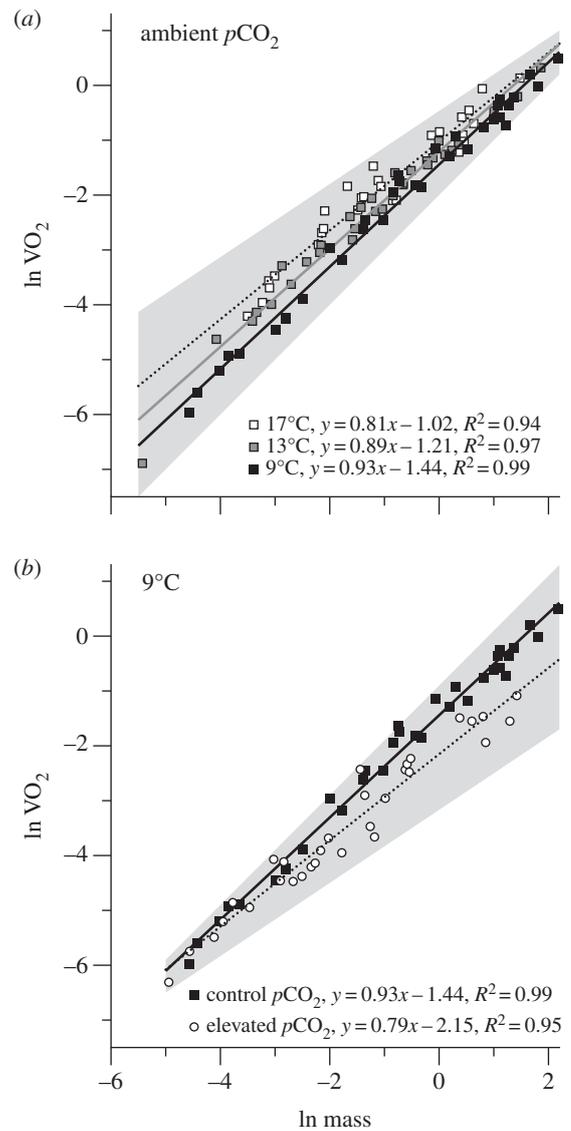
Figure 2. Sample linear models of ln VO$_2$ (mg O$_2$ h^{-1}) against ln mass (g, AFDM) from several *K. tunicata* treatments: (*a*) low (9°C), medium (13°C) and high (17°C) temperatures at control pCO$_2$; (*b*) control and elevated pCO$_2$ in low (9°C) temperatures. Shaded areas show the boundaries of $\frac{2}{3}$ and 1 between which metabolic scaling slope (**b**) may naturally vary, as proposed by several models. All other treatment plots and regression parameters are presented in the electronic supplementary material, figure S1 and table S1, respectively.

significantly decreased (*K. tunicata*: $F_{1,61} = 56.91$, $p < 0.001$; *M. muscosa*: $F_{1,53} = 13.24$, $p = 0.001$; figure 1*b*,*c*). The significant alteration to b along with metabolic depression again indicates an asymmetry in response across body-size; metabolic depression was much more pronounced in larger individuals (figure 2*b*).

This is the first study to examine metabolic scaling under the complex changes to carbonate chemistry associated with ocean acidification. A decrease in b was observed in a freshwater fish exposed to decreased pH (although a large difference of 3.5 units) [15], indicating a greater response by larger individuals. We similarly observed a decrease in b in two species under low pH (elevated pCO$_2$) and low temperatures, in combination with metabolic depression (figures 1*b*,*c* and 2*b*).

Although our treatments were designed within naturally experienced conditions, the present patterns could be a

consequence of a relatively short maintenance period. Physiological acclimation to altered conditions, where metabolism has reached a new state of equilibrium, can take weeks to months in a process governed by numerous factors, including cellular processes and mitochondrial density [16,17]. Asymmetric variation in acclimation rate or in the effects of the starvation period across body-size ranges could also affect apparent allometric relationships. Over several generations our observed patterns of changes to metabolic scaling may not be maintained, but revert to species-specific values in concert with transgenerational acclimation to altered conditions [18].

These alterations to metabolic allometry indicate that physiological responses to temperature and pH differ systematically with size and hence age. Such asymmetric responses, if preserved over several generations, could have implications for natural populations under warmer, more acidic future conditions. Both terrestrial and aquatic species may become smaller under environmental warming [19,20], and parameters such as reproductive capacity frequently vary with age; large mature individuals often provide a greater contribution to later generations [5]. Variable responses to physical conditions with age are also important because certain life stages are already under highly asymmetric ecological pressures [14].

In experimental physiology, some specific hypotheses necessarily focus on single cohorts or life stages, such as larvae, and thus a limited size range [14]. Studies on adults typically select narrow specimen size groups [5]. This allows for straightforward comparison of physiological metrics, but may assume equivalence in response among differently sized individuals. Confining our study only to larger individuals would have led to largely different conclusions; response to elevated pCO$_2$ would appear considerable (e.g. figure 2b) while responses to increased temperature would appear minor or non-existent (e.g. figure 2a). Neither of these gives an accurate portrayal of the complex overall species response, and experiments on limited size ranges may substantially over- or underestimate such responses. Our work adds to the growing evidence that body-size could be an important factor modulating species' physiological functioning in altered conditions under climate change.

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