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Temperature effects on mass-scaling exponents in colonial animals: a manipulative test

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Abstract. Body size and temperature are fundamental drivers of ecological processes because they determine metabolic rates at the individual level. Whether these drivers act independently on individual-level metabolic rates remains uncertain. Most studies of intraspecific scaling of unitary organisms must rely on preexisting differences in size to examine its relationship with metabolic rate, thereby potentially confounding size-correlated traits (e.g., age, nutrition) with size, which can affect metabolic rate. Here, we use a size manipulation approach to test whether metabolic mass scaling and temperature dependence interact in four species (two phyla) of colonial marine invertebrates. Size manipulation in colonial organisms allows tests of how ecological processes (e.g., predation) affect individual physiology and consequently population- and community-level energy flux. Body mass and temperature interacted in two species, with one species exhibiting decreased and the other increased mass-scaling exponents with increasing temperature. The allometric scaling of metabolic rate that we observe in three species contrasts with the isometric scaling of ingestion rates observed in some colonial marine invertebrates. Thus, we suggest that the often observed competitive superiority of colonial over unitary organisms may arise because the difference between energy intake and expenditure increases more strongly with size in colonial organisms.

Key words: energetics; latitude; metabolic theory; scaling; thermal limits.

Introduction

Metabolic theories seek to unite hierarchical levels of biological organization based on the scaling of energetic demands (i.e., metabolic rates), from individuals to ecosystems (Nisbet et al. 2000, Brown et al. 2004, van der Meer 2006). The role of individual body mass, M_i, as a determinant of individual-level metabolic rate, B_i, is key to this goal. Myriad studies have examined the relationship between B_i and M_i (Kleiber 1932, 1961, Peters 1983, Schmidt-Nielsen 1984, Glazier 2005, 2010, DeLong et al. 2010), typically revealing a power function of the form $B_i = B_o M_i^{\alpha}$, where B_o is a normalization constant independent of mass and α is a dimensionless scaling exponent. For most animals, B_i scales sublinearly (or negatively allometric, sensu Huxley 1932) with M_i, i.e., with $\alpha < 1$, generally falling between 0.5 and 1. Importantly, α estimates can also vary considerably among taxa and with many biotic and abiotic variables (e.g., Lovegrove 2000, Moses et al. 2008, White et al. 2009, 2012, DeLong et al. 2010, Killen et al. 2010, Kolokotrones et al. 2010, Vaca and White 2010, Glazier et al. 2011, Ketola and Kotiaho 2012, White and Kearney 2013, Barneche et al. 2014, Hirst et al. 2014). While these studies demonstrate clearly that the value of α is not fixed, most share a common limitation: they rely on preexisting differences in mass. Such correlative studies cannot address the causal association between B_i and M_i properly, because M_i is correlated with a range of other environmental, physiological, and life history variables (see e.g., Peters 1983, Calder 1984, Schmidt-Nielsen 1984 for comprehensive reviews) and many of these variables are also associated with B_i (Mcnab 2002, Konarzewski and Ksiazek 2013, White and Kearney 2013).

In contrast to studies that make use of intact individual organisms, colonial animals provide an opportunity for more definitive tests of the association between B_i and M_i. Colony size can be manipulated experimentally, hence allowing for the potential to remove the confounding effects of life-history traits that are also correlated with body size (e.g., Pratt 2005, Hart and Keough 2009, White et al. 2011). Furthermore, different colonial species may grow in different dimensions, and the mass-scaling exponent α is explicitly predicted to change with number of body dimensions (Nakaya et al. 2005, Ginzburg and Damuth 2008, White et al. 2011, Kearney and White 2012). For example, the fractal geometry model (West et al. 1997, 1999) predicts that $\alpha = n/(n + 1)$ for a n-dimensional organism, hence a value of $\alpha = 0.75$ is predicted for three-dimensional animals while a value of $\alpha = 0.67$ is predicted for two-dimensional animals (Savage et al. 2008, Enquist et al. 2009, Koontz et al. 2009, Banavar et al. 2010, Kolokotrones et al. 2010, but see Price et al. 2007 for additional predictions of α upon modifications of canonical assumptions). Currently, however, it is uncertain how colonial organisms may conform to these predictions because the original fractal

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geometry model considers resource-distribution networks within unitary organisms (or individual zooids) and not colonies that may (or may not) distribute resources in fundamentally different ways. Assessing the scaling of metabolic rate with body mass in colonial organisms is therefore a fundamental step in assessing how this mode of life conforms to general predictions, as well as providing an excellent opportunity for empirically manipulating mass.

Experimental size manipulation in colonial organisms also allows for tests of how ecological processes such as predation or disturbance affect individual physiology. For instance, it has been demonstrated that mimicking the effects of partial predation or physical disturbance through experimental size manipulation yields different responses in growth and reproductive onset in one arborescent (no effects on growth, delayed reproduction and reduced fecundity; Bone and Keough 2005) and one encrusting bryozoan (reduced growth, with reproductive onset and fecundity dependent on the age of remaining zooids; Hart and Keough 2009). On the other hand, size manipulations on one encrusting bryozoan (White et al. 2011) and one colonial ascidian (Nakaya et al. 2005) only affected the size of colonies, such that the scaling relationships between metabolic rate and colony size did not differ between size-manipulated and non-manipulated colonies. Sub-linear allometric scaling, in turn, implies that mass-specific metabolic rates decrease with size. Changes in size structure will therefore influence total population- and community-level respiration flux (if total standing biomass is held fixed; Allen et al. 2005, Barneche et al. 2014). Meanwhile, resource ingestion rates appear to scale linearly with size, in some colonial organisms at least (e.g., Pratt 2005), suggesting that these two key processes – energy ingestion and expenditure – scale very differently with colony size. These different scaling relationships suggest that larger colonies have an energetic advantage over smaller colonies, although this remains to be tested.

Metabolic rates are also influenced by temperature, typically following an exponential function (Gillooly et al. 2001, Kooijman 2009). Whether the temperature dependence and mass scaling of metabolic rates interact remains a contentious issue, though recent empirical evidence indicates that they do, and in various ways (e.g., Glazier 2005, 2010, 2014, Killen et al. 2010, Price et al. 2012). Testing for interactions between mass and temperature on metabolic rates matters because different mass-scaling exponents under different temperatures yield different metabolic efficiencies associated with different body sizes. For example, a body size that maximizes scope for reproduction under one temperature may be suboptimal in a cooler temperature or vice versa (Sebens 1987). In the present study, we experimentally manipulate size in different colonial marine organisms from two phyla (Bryozoa [Ectoprocta] and Porifera) in order to test whether the mass scaling and temperature dependence of metabolic rates interact (White et al. 2011, Kearney and White 2012). In doing so, we explicitly test if these interactions are general or species specific using a model selection procedure.

Methods

Animal collections

To collect our study organisms, roughened acetate sheets secured to the underside of 6 mm thick PVC sheets suspended from floating pontoons at a depth of 1 m were deployed at Manly Boat Harbour, Queensland, Australia (27°27′ S, 153°11′ E) and checked regularly to remove fouling organisms other than our species of interest: *Bugula neritina* and *B. stolonifera* (arborescent bryozoans that have a three dimensional tree-like growth form), *Hippopodina iririkiensis* (an encrusting bryozoan that grows mostly in two dimensions as flat disc), and an unidentified species of sponge belonging to the Microcionidae family (Hart and Marshall 2009, again a flat species that grows mostly in two dimensions; Table 1). After 6 weeks in the field (average SST 22°C), sheets bearing colonies were returned to the University of Queensland where they were kept in aerated seawater tanks (18°C) for up to 48 h prior to measurements. For encrusting colonies, sections of acetate bearing whole colonies were then cut from the sheets and fragmented (see e.g., Nakaya et al. 2005, White et al. 2011 for details and tests of potential experimental artifacts). Arborescent colonies were cut directly from the acetate sheets and fragmented. We focused on non-reproductive colonies of bryozoans (at that time of year 6 weeks was not sufficient time for colonies to show reproduction) to avoid the potentially complicating effects that reproduction might bring. For the sponge, we found no evidence of reproduction, though we cannot rule out sperm production.

Fragmentation procedures

For *Bugula neritina* and *B. stolonifera*, we reduced body size by cutting whole branches from the colony. This approach maintains the ratio of growing tips to core

Table 1. Mass ranges and sampling efforts for the four species of Bryozoa and Porifera experimentally manipulated in the present study at two extreme temperatures.

Species	10°C			25°C		
	Min. mass (mg)	Max mass (mg)	*n*	Min. mass (mg)	Max mass (mg)	*n*
Bugula neritina	3.5	286.8	70	6.0	391.5	76
Bugula stolonifera	1.1	65.3	34	0.7	92.2	55
Hippopodina iririkiensis	18.0	114.4	35	19.0	100.0	34
Microcionidae	10.3	189.8	45	11.7	359.7	52

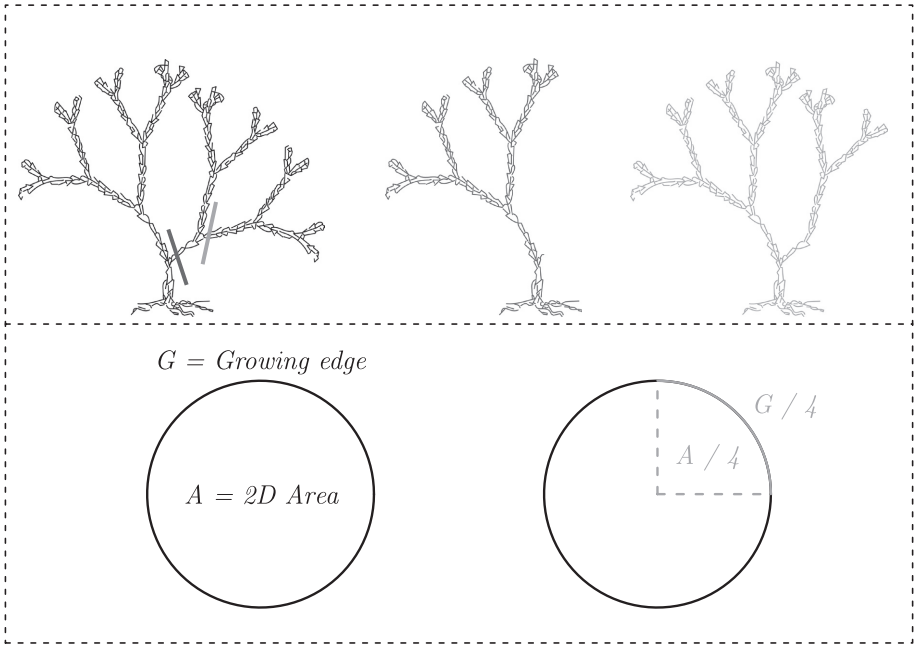

FIG. 1. Schematic illustration of our fragmentation approach. Top: Demonstration of how whole branches were cut (dark and light grey lines) in arborescent 3D bryozoans (*Bugula neritina* and *B. stolonifera*), and the resulting fragments (dark- and light-grey colonies) used for respirometry. Importantly, this approach maintains the ratio of growing tips to core zooids and importantly mimics the kind of natural size reductions that *Bugula* spp. experience in the field. Bottom: Approach adopted with encrusting 2D organisms (the bryozoan *Hippopodina iririkiensis* and the Microcionidae sponge), where, based on previous studies, pizza slices were used in order to maintain a constant ratio between individual growing edge (G) and area (A).

zooids (except for the base stolon present in all remaining fragments) and, importantly, mimics the kind of natural size reductions that *Bugula* spp. experience in the field when they are preyed upon by predatory nudibranchs (Bone and Keough 2005). For these arborescent species, we used just one fragment per colony because we always cut whole branches to manipulate size, leaving the base stolon intact (Fig. 1, top). For the encrusting species, we created "pizza slice" style fragments to maintain the ratio of outer growing edge to inner colony area (Fig. 1, bottom), which is important for growth of fragment colonies (Hart and Keough 2009, White et al. 2011). Importantly, the encrusting bryozoan used in this study has an extremely similar morphology and physiology to the one studied in White et al. (2011). Thus, it seems that artifacts are unlikely given that White et al. (2011) comprehensively demonstrated no differences in metabolic rates between intact and experimentally fragmented colonies of similar body size. We maintained the same edge-to-inner-ratio approach by cutting pizza slices for sponges. For the encrusting species, we used one to two fragments per colony.

We note that we did not record colony of origin so we cannot include "genotype" in our analyses. Importantly, our goal was not to partition variance in metabolic rate in a Genotype × Environment framework; instead, we were interested in how the phenotype of size influences metabolic rate under different temperatures. The nature of our fragmentation approach meant that the age profile of zooids within the fragments we created was identical, regardless of size, by cutting whole branches or pizza slices for arborescent and encrusting species respectively (Fig. 1). As such, age was not a confounding factor in the analysis as all colonies were approximately the same age, regardless of size.

Respiration measurements

Individual metabolic rates (converted to μL O_2 h^{-1}, n = 401), B_i, were measured as rate of oxygen consumption, $\dot{V}O_2$, of colony fragments at nominal temperatures of 10 or 25°C. All individuals (regardless of size) and species were randomly assigned to test temperatures, and we used replicate vials and controlled temperature cabinets (see below). Measurements were conducted using a 144-channel PreSens Sensor Dish Reader (SDR, AS-1 Scientific, Wellington, New Zealand) according to standard techniques (Köster et al. 2008, Lighton 2008, White et al. 2011). $\dot{V}O_2$ was measured by placing the colony fragment in a glass vial containing 0.2 μm filtered sea water and a non-consumptive O_2 sensor spot and calculated from the rate of change of O_2 saturation (m_a, % h^{-1}) as:

$$\dot{V}O_2 = -1\left(\frac{m_a - m_b}{100}\right) V\beta_{O_2}, \quad (1)$$

where m_b is the rate of change of O_2 saturation for blank vials containing no animals (% h^{-1}), β_{O_2} is the oxygen

capacitance of air-saturated sea water at 10°C (6.4 mL L^{-1}) or 25°C (4.74 mL L^{-1}) (Cameron 1986) and V is water volume (vials were 5 mL in volume and water volume was calculated by subtracting the volume of acetate and animals). At least four blank vials were recorded simultaneously with each 24-well plate to account for microbial oxygen consumption and sensor spots were calibrated with air-saturated seawater (100% A.S.) and water containing 2% sodium sulfite (0% A.S.). After a 1 h settling-in period (i.e., time necessary for the system to come into equilibrium), $\dot{V}O_2$ measurements lasted up to 20 h depending on individual mass (tiny individuals require much longer measurement periods in order to detect $\dot{V}O_2$). Following measurement of $\dot{V}O_2$, colony fragments were blotted dry and weighed to 0.1 mg (Sartorius A 200 S, Sartorius AG, Göttingen, Germany). All measurements of $\dot{V}O_2$ were made in one of four dark constant temperature cabinets, with temperatures within a run randomized among cabinets.

Statistical analyses

To test for the interacting effects of body mass and temperature on metabolic rate across species, we fitted a three-way interaction linear model using log-transformed data

$$\ln B_i \sim \ln M_i \times \text{Temperature} \times \text{Species}, \quad (2)$$

where mass, $\ln M_i$ (mg), is a continuous variable, while temperature (two levels: 10 and 25°C) and species (four levels corresponding to the four species) are categorical variables. With this model, we allow each species to have a different mass-scaling slope that may change with temperature; we also account for species- and temperature-specific deviations on the metabolic normalization (i.e., model intercept). We also included date of experiment, ΔD as a random effect on the metabolic normalization to help control for natural experimental variability. We then performed a model selection procedure (see below) by comparing the above full model with a series of nested two- and one-way interaction models in order to formally test for mass-temperature interactions in metabolic rates among different species.

All models were initially fitted using maximum likelihood (Zuur et al. 2009) with the R package *lme4* version 1.1-8 (Bates et al. 2015). Each nested model was compared against the full model using likelihood ratio tests, and models were considered significantly different if $P < 0.05$. We then fitted the best model in a Bayesian framework by calling *JAGS* version 3.4.0 from the R package *R2jags* version 0.05-03 (Su and Yajima 2015) in order to derive posterior distributions and associated 95% credible intervals (CIs) for the fitted parameters. Random effects were assumed to be normally distributed, with means of 0. Fitted parameters were assigned priors that were vague (i.e., locally uniform over the region supported by the likelihood) (Kruschke 2014). The posterior distributions of model parameters were estimated using Markov chain Monte Carlo (MCMC) methods by constructing three chains of 500,000 steps each, including 250,000-step burn-in periods. Chains were then updated with 500,000 steps each until convergence was reached using 250-thinning step interval, so a total of 6000 steps were retained to estimate posterior distributions (i.e., 3 × 500,000/250 = 6,000). All data and code necessary to reproduce this paper, its analyses, tables and figures can be obtained on GitHub https://github.com/dbarneche/MTRBrEs (Barneche et al. 2016*a*).

Results

The model selection indicated that the three-way interaction model (Eq. 2) performed significantly better than all nested two- and one-way interaction models (Table 2), thus implying that different species present different interactions between mass-scaling and temperature dependence. Particularly, the best model yields no interaction between mass scaling and temperature dependence for *Bugula stolonifera* and the Microcionidae sponge (large overlap between 95% credible intervals for the

Table 2. The best model was constructed, using the R package *lme4*, by successively removing fixed effects based on likelihood ratio tests of significance (Zuur et al. 2009). In the table, χ^2 and df refer to likelihood ratio test between the full model and nested model. The final best model, which includes all parameters, is indicated in bold.

Model	Predictors	df	χ^2	P
Full	**Species + lnMass + Temp + Species:lnMass + Species:Temp + Temp:lnMass + Temp:lnMass:Species**	-	-	-
M2	Full - Temp:lnMass:Species	3	9.95	**<0.05**
M3	Full - Temp:lnMass - Temp:lnMass:Species	4	11.95	**<0.05**
M4	Full - Species:Temp - Temp:lnMass:Species	6	56.26	**≤0.0001**
M5	Full - Species:lnMass - Temp:lnMass:Species	6	16.57	**<0.05**
M6	Full - Species:Temp - Temp:lnMass - Temp:lnMass:Species	7	56.30	**≤0.0001**
M7	Full - Species:lnMass - Temp:lnMass - Temp:lnMass:Species	7	18.80	**≤0.01**
M8	Full - Species:lnMass - Species:Temp - Temp:lnMass:Species	9	62.53	**≤0.0001**
M9	Full - Species:lnMass - Species:Temp - Temp:lnMass - Temp:lnMass:Species	10	62.64	**≤0.0001**

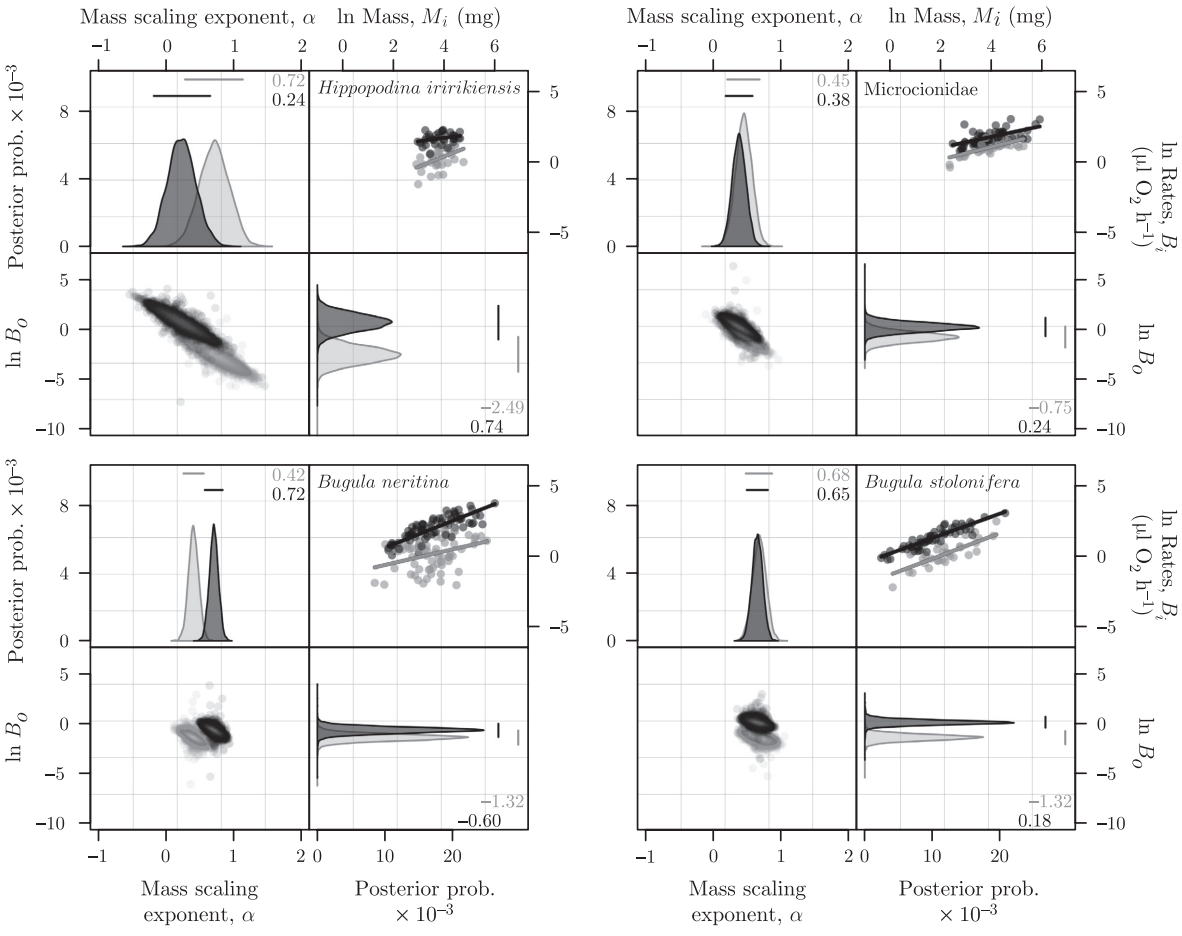

FIG. 2. Estimates from the three-way interaction model as obtained by Bayesian methods in *JAGS*. Results for each species are represented in groups of four plots, with grey and black colors representing actual data and parameter estimates at 10 and 25°C, respectively. *Top-left* and *bottom-right*: posterior probabilities of mass-scaling exponents and metabolic normalizations (respectively), with bars over curves representing 95% credible intervals, and numbers representing average estimates. *Bottom-left*: covariance between mass-scaling exponents and metabolic normalizations for each of the 10,000 MCMC iterations (translucent circles), with overlaid density heat-map. *Top-right*: regressions of individual metabolic rate, $\ln B_i$, as functions of individual body mass, $\ln M_i$. Regression fits were plotted using the average from the posterior distributions. In probability posterior plots (*top-left* and *bottom-right*), smooth probability curves (n = 511 values) were approximated from posterior probability density curves (n = 512 values); differences between adjacent x values (i.e., parameter estimates) were multiplied by the average of adjacent y values (i.e., respective probability density values; $(x_{i+1}-x_i) \times ((y_{i+1}-y_i)/2)$ thus insuring that the integral of the probability curve ≈1.

mass-scaling slopes, α) (Fig. 2). Still, while the mass-scaling slopes are statistically the same between temperatures for these species, there is evidence for higher metabolic normalizations (i.e., intercepts), B_o, at higher temperatures (though marginally significant for Microcionidae, i.e., slight overlap between 95% credible intervals; bottom-right quadrants in Fig. 2). For example, metabolic rates increase 4.5-fold between 10 and 25°C for *B. stolonifera*, yielding a Q_{10} of 2.7, which is equivalent to an activation energy of 0.73 eV (Gillooly et al. 2001, Yvon-Durocher et al. 2012). The best model indicates that the difference in metabolic rates between 25 and 10°C for *Hippopodina iririkiensis* decreases, on average, 4.3-fold moving from 7 to 148 mg. For *Bugula neritina*, however, rates increase 0.8-fold along the same body-mass gradient.

Average posterior estimates of mass-scaling exponents (top-left quadrants in Fig. 2) are inconsistent among the studied species and between growth forms (2D and 3D). For example, for organisms that exhibit 2D growth, *Hippopodina iririkiensis* shows exponents that encompass both 0.67 (Fractal geometry model prediction for 2D organisms; Koontz et al. 2009) and 0.75 (Fractal geometry model prediction for 3D organisms; West et al. 1997, 1999) at 10°C (95% CI: 0.29–1.14), but that are significantly shallower than 0.75 at 25°C (95% CI: −0.17–0.66). Microcionidae presents exponents that only overlap 0.67 at 10°C, but not at 25°C (95% CI at 10°C: 0.21–0.69; 95% CI at 25°C: 0.19–0.58). On the other hand, for 3D organisms, *Bugula neritina* presents scaling exponents that overlap both 0.67 and 0.75 at 25°C (95% CI: 0.58–0.84), but are significantly shallower at 10°C (95% CI:

0.27–0.57). Finally, *Bugula stolonifera* presents values that overlap both 0.67 and 0.75 at both temperatures (95% CI at 10°C: 0.48–0.87; 95% CI at 25°C: 0.50–0.81).

Discussion

Here, we show experimentally that the mass-scaling of metabolic rates may change with temperature, and in different ways across different species of marine invertebrates and between growth forms. We thus add to recent empirical evidence documenting mass-temperature interactions in metabolic rates (e.g., Glazier 2005, Killen et al. 2010). Possibly, the ability of our studied species to acclimate upon acute temperature changes could have affected the results (see discussion in Glazier 2014). Our experimental procedure, however, randomized species and individual sizes across temperatures such that systematic effects across sizes or among species are unlikely. Moreover, the temperature ranges span those experienced by these species in the field. Still, if mass-temperature interactions are proven to be frequent and consistent across different types of organisms, future studies will be necessary to mechanistically explain the interactive effects between mass scaling and temperature dependence of biological rates (but see the metabolic-level boundaries hypothesis in Glazier 2005, 2010, 2014).

That metabolic rate scales at an exponent of <1 across all four species (with the exception of *Hippopodina iririkiensis* measured at 10°C) has interesting implications for our understanding of the energetics of growth for different colonial animals. For instance, evidence suggest that water clearance and feeding rates scale either isometrically or allometrically with colony size in sponges (Riisgard et al. 1993, Kowalke 2000, McMurray et al. 2014), and these rates may follow the same mass scaling of metabolic rates (see e.g., Thomassen and Riisgard 1995). In contrast, most studies indicate that feeding rates scale isometrically or superlinearly in both encrusting and arborescent bryozoans (e.g., Okamura 1984, 1985, Pratt 2005). Our results and those from other studies (Hartikainen et al. 2014) indicate that metabolic rates scale allometrically in arborescent 3D bryozoans. Consequently, while every unit increase in size results in proportional increase in feeding rate, every unit increase in size results in a less-than-proportional increase in metabolic rate. Thus, as colonies increase in size, their capacity to capture food increases more quickly than the rate at which they expend energy.

The difference between the mass-scaling of ingestion rates and metabolic rates may explain the exponential growth and reproduction observed in some colonial marine invertebrates (e.g., Padilla et al. 1996, Winston 2010, Marshall and Monro 2013) – because the ratio of intake to expenditure increases with size, larger colonies can allocate proportionately more energy to growth and/or reproduction. In other words, for these colonial marine invertebrates, larger colonies are more efficient at garnering food and allocating it to somatic growth and/or reproduction than smaller colonies. Isometric energy gains and allometric energy expenditure in colonial marine invertebrates may explain why they are often competitively dominant, outgrowing and excluding unitary organisms (which generally present allometric scaling of both feeding and metabolic rates, see e.g., Kooijman 2009, Kearney and White 2012) in a range of systems (Buss 1980, 1990). It also suggests that perhaps colony- and population-level energy use increase with individual body mass, rather than showing energetic equivalence (Damuth 1987). Importantly, if that is the case, this scenario would add a new mechanism to explain deviations from energetic equivalence, which are thought, for example, to be a consequence of size-dependent mortality rates in eusocial insects (DeLong 2011) and energetic subsidies in reef fishes (Barneche et al. 2016*b*).

Although the mass scaling exponent was unaffected by temperature for two species (*Bugula stolonifera* and Microcionidae), it decreased and increased for one encrusting (*Hippopodina iririkiensis*) and one arborescent species (*Bugula neritina*), respectively. These results imply that energy use may vary for the same species in different environments, possibly affecting their ultimate colonization success and abundance. For example, the fact that *Bugula neritina* presented a shallower mass-scaling exponent at colder temperatures possibly indicates that large cold-environment individuals are more efficient in spending energy than counterparts in warm environments. On the other hand, our results indicate that *Hippopodina iririkiensis* presents a reduction in metabolic benefits of attaining a larger size at colder temperatures. Importantly, though, we note that there are many extrinsic factors that may affect abundance and selection on body size (e.g., predation, competition). Nonetheless, such differentials in the costs and benefits of attaining large size may help explain why some species living in cold temperatures present a slower pace of life (e.g., Rosa and Seibel 2010). Moreover, incorporation of differences in the size-dependence of the costs and benefits of size might improve current attempts to scale up energy from individuals to higher levels of organization (e.g., Allen et al. 2005, Yvon-Durocher and Allen 2012, Barneche et al. 2014, 2016*b*).

In the wild, partial predation and physical disturbance are common sources of depression of metabolic rates in unitary organisms (e.g., Glazier et al. 2011) and partial mortality in colonial organisms (Sebens 1987). Our findings suggest that, for some colonial species, size reductions mediated by predation or disturbance might modulate the energetic demands of individuals of similar body mass in different environments, thus affecting life-history trajectories. For instance, at 25°C, a 400-g individual of *Bugula stolonifera* will spend ~4-fold more energy than a similar-sized individual at 10°C. Conversely, due to the estimated positive interaction between mass-scaling and temperature, a similar-sized individual of *B. neritina* will spend ~12-fold more energy at 25°C than at 10°C. These differences, for instance, may help determine which species is a better competitor in different

environments, as individual metabolic rates will also affect growth rates and time of reproduction onset (Sebens 1987, Hart and Keough 2009).

At the population level, disturbance and predation can also create different size distributions in colonial organisms. Our finding of allometric scaling of metabolic rate implies non-intuitive outcomes of such shifts in mean size of colonies in a population. For example, at 25°C a population of 10 colonies of Microcionidae that each weigh 400 mg would have a cumulative metabolic demand of ~128 $\mu L\ O_2\ h^{-1}$. If a disturbance event halved each colony with minimal loss of biomass, then a population of those (now) 20 colonies (i.e., density twice as high), each weighing 200 mg, would have cumulative metabolic demand that is 1.5-fold higher (i.e., ~196 $\mu L\ O_2\ h^{-1}$), according to their new size. Thus, disturbance events change not only the size distribution of colonies but also increase the energy requirements of the population even if total standing biomass remains unchanged (Barneche et al. 2014). This new predation-mediated density dependence effect will increase total population energy flux, which is opposite to what is observed in unitary organisms that form groups or colonies, where group- or population-level metabolic rates often decrease due to increasing intraspecific competition (e.g., DeLong et al. 2014). We note, however, that a "colony" here is a collection of physiologically connected interdependent zooids, which is different from a colony of independent unitary individuals, hence different mechanisms are likely to be operating as density of individuals changes.

Here we have demonstrated that the effects of temperature can interact in different ways with the mass-scaling of metabolic rates in different species of colonial marine invertebrates encompassing two distinct phyla. By adopting a size-manipulation approach, we achieved a more definitive test of mass-scaling of metabolic rates by eliminating confounding effects of age and other functional traits that could also be correlated with size and/or metabolic rates. Mass-scaling exponents vary for different species, and are not consistent across growth forms nor phylogeny. Finally, our study suggests that the often observed competitive superiority of colonial organisms over unitary ones may arise because energy availability for growth and or reproduction increases more strongly with size in colonial organisms.

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Data Availability

All data and code necessary to reproduce this paper, its analyses, tables and figures can be obtained on GitHub https://github.com/dbarneche/MTRBrEs (Barneche et al. 2016*a*, doi: 10.5281/zenodo.159736).