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Author(s): Craig R. White, Michael R. Kearney, Philip G. D. Matthews, Sebastiaan A. L. M. Kooijman, and Dustin J. Marshall
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A Manipulative Test of Competing Theories for Metabolic Scaling

Craig R. White,[1,*] Michael R. Kearney,[2] Philip G. D. Matthews,[1] Sebastiaan A. L. M. Kooijman,[3] and Dustin J. Marshall[1]

1. School of Biological Sciences, University of Queensland, St. Lucia, Queensland 4072, Australia; 2. Department of Zoology, University of Melbourne, Melbourne, Victoria 3010, Australia; 3. Department of Theoretical Biology, Free University, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

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ABSTRACT: The reasons why metabolic rate (B) scales allometrically with body mass (M) remain hotly debated. The field is dominated by correlational analyses of the relationship between B and M; these struggle to disentangle competing explanations because both B and M are confounded with ontogeny, life history, and ecology. Here, we overcome these problems by using an experimental approach to test among competing metabolic theories. We examined the scaling of B in size-manipulated and intact colonies of a bryozoan and show that B scales with $M^{0.5}$. To explain this, we apply a general model based on the dynamic energy budget theory for metabolic organization that predicts B on the basis of energy allocation to assimilation, maintenance, growth, and maturation. Uniquely, this model predicts the absolute value of B, emphasizes that there is no single scaling exponent of B, and demonstrates that a single model can explain the variation in B seen in nature.

Keywords: allometry, metabolic rate, dynamic energy budget, metabolic theory of ecology.

Introduction

Understanding the factors that determine the metabolic rate of animals has been a key focus of physiological ecology for more than 100 years (Rubner 1883; Kleiber 1932; Patterson 1992; West et al. 1997, 1999; Kozłowski et al. 2003; Savage et al. 2004; White and Seymour 2004; White et al. 2007; McNab 2008; Glazier 2010; Isaac and Carbone 2010; Kolokotrones et al. 2010; Kooijman 2010). The relationship between metabolic rate (B) and body mass (M) is one of the most studied in biology; this relationship is notable because B is not proportional to M. Instead, B scales allometrically according to a power function $B = aM^{\beta}$, where β is typically less than 1. For much of the past century, β was thought to be approximately 0.75 (Kleiber 1932; Savage et al. 2004). This belief led to a search for a universal explanation for β, which was applicable from unicellular organisms to the largest mammals (e.g., West et al. 1997, 1999). Recent analyses have challenged this view, highlighting that no single scaling exponent is appropriate for all animals (Kozłowski and Konarzewski 2004; Chown et al. 2007; O'Connor et al. 2007; White et al. 2007; Glazier 2010; Isaac and Carbone 2010; Kolokotrones et al. 2010). Consequently, there is a need to understand not only why β is often less than 1 but also why β varies (Kozłowski et al. 2003; Glazier 2010). A problem with previous analyses is that B and M are measured in intact organisms and the resultant correlation is assessed, meaning it is typically impossible to determine the causal effect of M on B through experimental manipulation. When applied to the analysis of allometric scaling, correlational approaches are problematic because many variables in addition to B covary with body mass; in mammals, for example, body mass is correlated with climate, diet, and life-history traits, including litter size and maximum longevity (McNab 2008; Jones et al. 2009), all of which have been shown to have confounding effects on B (e.g., White and Seymour 2004; McNab 2008).

Distinguishing among competing explanations for β is made difficult because many models predict similar values. Correlational approaches to comparing alternative theories deliver only weak inference because more than one mechanism can often produce any given set of data (McGill 2003). For example, β may be less than 1 because of the fractal-like design of exchange surfaces and distribution networks (West et al. 1997, 1999). A definitive test of this fractal geometry model has proven elusive, however, because like several other models (e.g., Banavar et al. 1999, 2002), it predicts a central tendency of 0.75-power scaling (West et al. 1997, 1999). Thus, while this prediction shows good general agreement with the mean scaling exponent

* Corresponding author; e-mail: craig.white@uq.edu.au.

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observed in both intraspecific (Moses et al. 2008) and interspecific (Savage et al. 2004) correlational studies, other models make similar predictions. Models based on variation in cell size with body size (Kozłowski et al. 2003) and metabolic boundaries (Glazier 2010) both predict β to vary between 0.67 and 1, the midpoint of which is also close to 0.75. Similarly, the metabolic level boundaries (Glazier 2010) and dynamic energy budget (DEB; Kooijman 2010) models both predict that scaling exponents for resting endotherms will be lower than those for resting ectotherms. Such proximity among predictions hampers definitive tests. However, it has previously been suggested that examining the scaling of metabolic rate in two-dimensional organisms, such as flatworms and colonial ascidians and bryozoans, may offer a potential solution to this problem (West et al. 1997, 1999; Nakaya et al. 2005). For an n-dimensional organism, the fractal geometry model predicts that β should be $n/(n + 1)$, so for two-dimensional organisms, a value of $\beta = 0.67$ is predicted (West et al. 1997, 1999; see also Koontz et al. 2009). Recent modifications of the fractal geometry model predict values that encompass the range 0.5–1.0 (e.g., Price et al. 2007), with the exact value dependent on a range of assumptions (Enquist et al. 2007; Savage et al. 2008; Banavar et al. 2010; Kolokotrones et al. 2010). The predicted scaling exponent for an organism with a plane-filling network and area-increasing branching is 0.86 (Price et al. 2007), so the fractal model predicts an exponent of either 0.67 (West et al. 1997, 1999) or 0.86 (Price et al. 2007) for two-dimensional animals, depending on the assumptions used. Although these resource distribution models can predict a wide range of scaling exponents, they all retain the core property that resources flow from a central source (Banavar et al. 2010). Alternative models that are not based on resource distribution from a central source predict a similar range of exponents. The space-lifetime hypothesis (Ginzburg and Damuth 2008) suggests that organismal scaling should sometimes include a temporal dimension (e.g., generation time), in addition to the spatial ones usually considered, such that an exponent of $n/(n + 1)$ is predicted for n-dimensional organisms (i.e., an exponent of 0.67 for an organism with two spatial dimensions). Models based on mass transfer of metabolically important compounds (Patterson 1992) and self-organized criticality (Nakaya et al. 2005) predict exponents of 1.1–1.25 and 0.75, respectively, and DEB models (Kooijman 2010) predict isometric scaling if horizontal transport of metabolites is fast relative to growth and an exponent of 0.5 if horizontal transport is slow relative to growth. Two-dimensional organisms therefore provide a definitive test of metabolic theory, yet such examinations remain rare (Nakaya et al. 2005).

In this study, we overcome the limitations of previous correlational studies on three-dimensional organisms by using a manipulative approach on a two-dimensional organism. We focused on colonies of the encrusting bryozoan *Hippoporina indica*; these colonies are composed of multiple individual zooids derived from single ancestrula. Only zooids at the perimeter bud new zooids, producing a two-dimensional morphology that can be modeled as a single organism: resources required for growth are gathered by all members of the colony and distributed via a network of communication pores, resulting in colony-wide resource integration (Miles et al. 1995). We examine the effect of experimentally manipulating the size of colonies on β and compare observed values with those explicitly predicted by the range of models of metabolic scaling. In contrast to other studies of two-dimensional colonial animals that report scaling exponents between 0.75 and 1.125 (Hughes and Hughes 1986; Muñoz and Cancino 1989; Hunter et al. 1996; Nakaya et al. 2003, 2005; Peck and Barnes 2004), we found a scaling exponent of 0.5. To explain this variability, we developed a model based on the DEB theory specifically for two-dimensional organisms. Our derived model successfully predicted our findings as well as the variable estimates of β generated from other studies.

Material and Methods

To collect colonies for study, we deployed roughened acetate sheets secured to the underside of 6-mm-thick PVC sheets suspended from floating pontoons at a depth of 1 m 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, *Hippoporina indica*. After 6 weeks in the field, acetate sheets bearing colonies of *Hippoporina* were returned to the University of Queensland, where they were maintained in aerated seawater for up to 48 h before measurements. Small sections of acetate bearing whole colonies were then cut from the sheets and left either whole or fragmented, depending on the experiment (fig. 1). Colony fragments were produced by cutting sections from larger colonies with a scalpel blade (fig. 1). We used experimentally manipulated fragments rather than whole colonies because we wanted to exclude all potentially confounding effects that are often associated with increased size and focus on the effects of size alone. We were concerned that fragments of different sizes would also differ in their perimeter : area ratio and therefore the ratio of damage that they experienced relative to their body size. To test for any damage effects, we therefore performed a control study whereby we used the same experimental techniques but trimmed a one-zooid-thick annulus from the perimeter of small colonies of varying size (fig. 1). We then compared the metabolic rate of intact and edge-

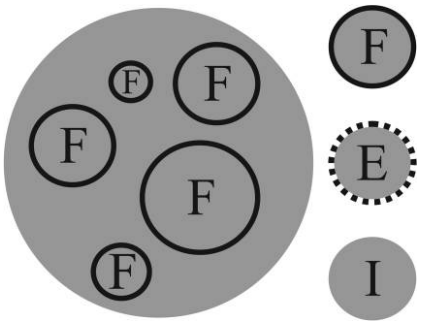

Figure 1: Schematic diagram of colony fragmentation procedures. Colony fragments (F) were produced by cutting large intact colonies into pieces of a range of sizes. Edge-manipulated colonies (E) were produced by removing a one-zooid-thick ring from the edge of small intact colonies (I). Small intact colonies served as a control for testing the effects of damage on metabolic rate.

manipulated colonies and found no effects of damage on colony respiration rate (see "Results"), suggesting that we can exclude damage as a potential explanation for our findings.

The rate of oxygen consumption ($\dot{V}O_2$) of whole colonies and colony fragments was measured at 24°C using a 48-channel PreSens sensor dish reader (SDR; AS-1 Scientific, Wellington, New Zealand), according to standard techniques (Alton et al. 2007; Köster et al. 2008; Lighton 2008). The $\dot{V}O_2$ was measured by placing the colony or fragment in a glass vial containing 0.2 μm filtered seawater and a nonconsumptive O_2 sensor spot and calculated from the rate of change of O_2 saturation (m_a; % h^{-1}) as

$$\dot{V}O_2 = -1\frac{m_a - m_b}{100}V\beta O_2,$$

where m_b is the rate of change of O_2 saturation for blank vials containing no bryozoans (% h^{-1}), βO_2 is the oxygen capacitance of air-saturated seawater at 24°C (4.88 mL L^{-1}; Cameron 1986), and V is water volume (chambers were 0.005 or 0.025 L, and water volume was calculated by subtracting the volume of acetate and animals). At least four blank vials were recorded simultaneously with each run to account for microbial oxygen consumption, and sensor spots were calibrated with air-saturated (AS) seawater (100% AS) and water containing 2% sodium sulfite (0% AS). After measurement of $\dot{V}O_2$, colonies or fragments were blotted dry and weighed to 0.1 mg (Sartorius A 200 S, Sartorius, Göttingen, Germany). All measurements of $\dot{V}O_2$ were made in a dark constant-temperature cabinet (SEM RI90-DOP, ProSciTech, Thuringowa, Australia), and water within the vials on the SDR plates was mixed gently throughout measurements, using an orbital mixer (Ratek OM1, Ratek Instruments, Boronia, Australia). The scaling exponent (β) of the relationship between $\dot{V}O_2$ and fragment mass (M) was then estimated by ordinary least squares (OLS) linear regression of $\log(\dot{V}O_2)$ on $\log(M)$ and compared with the explicit predictions of the fractal geometry model ($\beta = 0.67$; West et al. 1997, 1999), the mass transfer model ($\beta = 1.1$–1.25; Patterson 1992), the self-organized criticality model ($\beta = 0.75$; Nakaya et al. 2005), the space-lifetime hypothesis ($\beta = 0.67$; Ginzburg and Damuth 2008), and a prediction for the value of β based on a model developed according to the principles of the DEB model (Kooijman 2010).

OLS regression was used in preference to reduced major axis regression (RMA) or nonlinear regression of untransformed data because we estimate that the measurement error in determination of mass is considerably less than one-third of that in determination of $\dot{V}O_2$ (McArdle 1988, 2003) and because growth in bryozoans is multiplicative rather than linear, thereby making log transformation of M and $\dot{V}O_2$ appropriate (Finney 1989; Kerkhoff and Enquist 2009; White 2011). For comparative data, a number of recent studies have advocated a range of alternatives to OLS and RMA line-fitting procedures (e.g., Isler et al. 2002; Hayes and Schonkwiler 2006; Warton et al. 2006; Hui and Jackson 2007; O'Conner et al. 2007, among others). In this study, we eschew these approaches in favor of OLS regression because, in contrast to correlational analyses of comparative data, in this study the independent variable (M) is manipulated and we test for an effect of this manipulation on $\dot{V}O_2$.

Results

The scaling exponent of $\dot{V}O_2$ did not differ between intact colonies, edge-manipulated colonies, and colony fragments of *Hippoporina* (ANOVA, $F_{2,153} = 0.11$, $P = .90$); $\dot{V}O_2$ was significantly positively related to mass (ANCOVA, $F_{1,155} = 86.3$, $P < .0001$) and differed between intact colonies, edge-manipulated colonies, and colony fragments (ANCOVA, $F_{2,155} = 11.6$, $P < .0001$). The elevation of the relationship between $\dot{V}O_2$ and mass did not differ significantly between intact and edge-manipulated colonies, but the elevation of the relationship between $\dot{V}O_2$ and mass of colony fragments was lower than that of intact and edge-manipulated colonies (Tukey's HSD). The scaling exponent of $\dot{V}O_2$ for 137 colony fragments ranging in size from 7.9 to 194.9 mg was ~0.5 and differed significantly from 0.67, 0.75, and 1 (fig. 2).

A Model for the Scaling of Metabolic Rate in Colonial Organisms

We found that the metabolic scaling exponent in our study did not match the predictions of most of the extant models

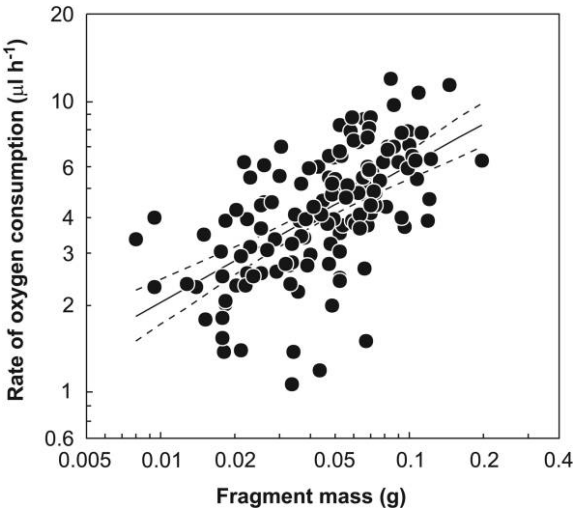

Figure 2: Allometric scaling of rate or oxygen consumption in bryozoan colony fragments. The relationship is statistically significant, and the scaling exponent (0.47 ± 0.11, 95% confidence interval [CI]) is significantly different from 0.67, 0.75, and 1. Dashed lines are the 95% CI of the regression.

of metabolism. Furthermore, when we compared our findings with those of the few other studies on two-dimensional organisms, estimates of β varied from 0.75 to 1.125 (Hughes and Hughes 1986; Muñoz and Cancino 1989; Hunter et al. 1996; Nakaya et al. 2003, 2005; Peck and Barnes 2004). We therefore explored whether a version of the DEB model, derived explicitly for two-dimensional organisms, to incorporate parameters for which data are available, could successfully account for our finding as well as the variability observed in other studies.

To estimate the metabolic rate of a colony, we first consider the metabolic rate of an individual zooid, using general principles that apply to any animal (Kooijman 2010). In such a framework, metabolic rate is considered to comprise a weighted sum of four processes: assimilation, maintenance, growth, and maturation. Since in this study metabolic rate was measured in filtered seawater (i.e., with little or no food available and measured after sufficiently long to ensure clearance of the gut), we consider only growth and maintenance (note, however, that the model predictions are robust to an increase in maintenance costs such as would be observed during assimilation; see appendix, available online). Growth includes an overhead cost, so most of the use of O_2 associated with growth concerns the overhead costs of growth. In most encrusting bryozoans, including the species discussed here, only zooids at the edge grow (Hart and Keough 2009), so the total metabolic rate of a bryozoan colony, B, consists of the metabolism of the edge B_e and the center B_c. The metabolic rate of a growing zooid at the colony edge, b_e, includes metabolism attributable to maintenance γ_m and growth γ_g ($b_e = \gamma_m + \gamma_g$), whereas the metabolic rate of a nongrowing zooid at the center of the colony, b_c, includes only maintenance ($b_c = \gamma_m$).

If we assume a circular colony of uniform thickness d composed of a single layer of rectangular zooids arranged perpendicular to the colony perimeter with width equal to d, B is calculated as

$$B = b_e \frac{\pi(D/2)^2 - \pi[(D-l)/2]^2}{dl} + b_c \frac{\pi[(D-l)/2]^2}{dl}, \quad (1)$$

where D is colony diameter and l is the length of an individual zooid. Since the mass of an individual zooid, m, can be calculated from its volume d^2l and density ρ and a colony is one zooid thick, it follows that the mass of a colony, M, can be calculated as $\rho d\pi(D/2)^2$, so $D = 2(M/\rho d\pi)^{0.5}$. Assuming that d, l, and ρ are independent of M (i.e., are proportional to M^0), $D \propto M^{0.5}$ and equation (1) can be rewritten as

$$B \propto b_c M + (b_e - b_c) M^{0.5}. \quad (2)$$

Equation (2) predicts that B scales isometrically with colony mass when $b_e = b_c$, but when $b_e \gg b_c$, the scaling exponent for B approaches 0.5 (for a detailed derivation of the model for the general case of encrusting organisms, using the formal DEB parameters and state variables, see http://www.bio.vu.nl/thb/research/bib/Kooy2010_c.pdf). Since γ_g is calculated as the product of growth rate r_g and a mass-specific cost of growth c_g, it follows that $b_e/b_c = (\gamma_m + c_g r_g)/\gamma_m$. The ratio b_e/b_c increases with r_g since c_g and r_g are independent (Wieser 1994), so the scaling exponent of B is predicted to be close to 0.5 in fast-growing colonies but close to 1 in slow-growing colonies.

To parameterize the model, the maintenance metabolic rate of nongrowing zooids was assumed to be equivalent to the lower limit of the range of the mean mass-specific metabolic rates observed in more than 3,000 species (1 W kg^{-1} dry mass at 25°C, based on an observed range of 0.3–9 W kg^{-1} for species with an average water content of 70%; Makarieva et al. 2008). The temperature dependence (Q_{10}) of maintenance metabolic rate was assumed to be 2, which is similar to that reported for other animals (Makarieva et al. 2008). Overhead costs of dry mass growth were estimated at 8.4 J mg^{-1} for tissue (Hou et al. 2008) and 1.5 J mg^{-1} for inorganic calcification (Palmer 1992); this mean value for the overhead cost of tissue synthesis falls within the range of 4.3–16.5 J mg^{-1} presented elsewhere (Palmer 1992; Wieser 1994). Zooid dry mass and water content were estimated as 3.92×10^{-5} g and 84%, respectively (Stebbing 1971), and the dry mass of zooids was estimated to be 39% organic (Peck and Barnes 2004).

Growth rates of intact colonies were estimated to be 33 cm $year^{-1}$ for temperate species (Marshall and Keough 2004, 2008; Hart and Keough 2009). Recently manipulated colony fragments grow at a rate equal to ~45% of similarly sized intact colonies (Hart and Keough 2009), or 15 cm $year^{-1}$ for colony fragments, and recently edge-manipulated colonies grow at ~70% of intact colonies (Hart and Keough 2009). Zooid dimensions were estimated at 1.014 × 0.575 × 0.575 mm and were based on measurements made under a dissecting microscope. Metabolic rates were converted to rates of oxygen consumption, assuming an energy equivalence of O_2 of 20.08 J mL^{-1} (Withers 1992; Schmidt-Nielsen 1997; McNab 2002; Lighton 2008). Sensitivity analyses demonstrate that prediction of the scaling exponent is robust to variation in input parameters over a range of at least several fold (fig. A3, available online), and prediction of the elevation of the scaling relationship is most sensitive to variation in zooid dimensions (fig. A4, available online).

Discussion

Contrary to the predictions of all except the DEB theory (Kooijman 2010), we find that the scaling exponent of $\dot{V}O_2$ (a proxy for metabolic rate, B) for encrusting bryozoans is ~0.5 (fig. 2). The allometric scaling of colony metabolism with mass does not arise as a consequence of metabolic depression associated with oxygen limitation of the largest colonies or fragments because $\dot{V}O_2$ is independent of the partial pressure of oxygen (PO_2) to at least 3 kPa, and the lowest PO_2 measured in still water immediately adjacent to an intact colony was 7.2 ± 0.6 (SD) kPa (appendix).

Our finding that smaller colonies have much greater energy expenditure for a given mass relative to larger colonies ($\beta = 0.5$ cf. ~0.75 in many animals) has interesting consequences for the ecology and evolution of encrusting colonial organisms (such as bryozoans, corals, and ascidians). If the relationship between energy intake and colony size scales with an exponent greater than that of colony metabolism (as might be expected, given that the number of feeding modules is strongly related to colony size; Sebens 1987), then the mismatch between energy gains and losses will be far greater for large colonies. The mismatch between energy gains and losses in larger colonies may therefore be the mechanism responsible for the increased relative growth rates of larger colonies (Hart and Keough 2009). While ecologists have long recognized the ecological benefits of increased colony size for modular organisms (e.g., increased competitive ability and predation resistance; Sebens 1987; Buss 1990; Hart and Keough 2009), our study suggests that increases in colony size also carry metabolic benefits—attaining a larger size enables colonies to be more efficient in their use of resources. If increased size carries metabolic benefits, decreases in size must carry metabolic costs. Physical disturbance often causes the fragmentation of bryozoan colonies, and the level of physical disturbance that colonies experience varies among populations and habitats (Hart and Keough 2009). Our results suggest that populations of bryozoans experiencing higher rates of fragmentation will have greater energy requirements than populations that experience lower rates of fragmentation. Thus, changes in disturbance regimes could affect the productivity of populations in previously unanticipated ways. Disturbance could change the energy requirements of populations even in the absence of shifts in population biomass via shifts in size structure and thus mass-specific metabolic rate.

Explaining Variation in Metabolic Scaling: Predictions Based on the DEB Model

Our modified version of the DEB model has predictive power across environments, species, and phyla. Previous studies on other colonial marine invertebrates report a β value between 0.75 and 1.125, and most models cannot explain this variability (Hughes and Hughes 1986; Muñoz and Cancino 1989; Hunter et al. 1996; Nakaya et al. 2003, 2005; Peck and Barnes 2004). Models of resource transport through outward-directed distribution networks (West et al. 1997, 1999; Banavar et al. 1999, 2002, 2010) predict scaling exponents between 0.5 and 1.0, depending on network geometry (e.g., Price et al. 2007), but such models require that resources are distributed from a single source (e.g., Banavar et al. 2010). Given that resources required for growth are gathered by all members of a bryozoan colony and distributed throughout the colony via a network of communication pores (Miles et al. 1995), models of outward-directed nutrient transport from a single source are inappropriate for these animals.

The DEB model predicts that the energy expenditure of colony edges differs from that of colony centers such that, uniquely, the model predicts that the scaling exponent of metabolic rate (β) should vary among species with different growth rates. The diameter of bryozoan colonies from temperate areas increases linearly with time at a rate 50–80 times faster than that of Antarctic species (~33 and 0.5 cm $year^{-1}$, respectively; Barnes and Clarke 1998; Barnes and Arnold 2001; Marshall and Keough 2004, 2008; Hart and Keough 2009), and so β is predicted to vary among environments. For *Hippoporina*, β is predicted to be 0.5, as shown in this study (fig. 2), and for slower-growing Antarctic species, the model correctly predicts isometric scaling (i.e., $\beta = 1$; fig. 3; Peck and Barnes 2004). Interestingly, the bryozoan *Electra pilosa* grows at a rate similar to that of *Hippoporina* (Hughes and Hughes 1986; Her-

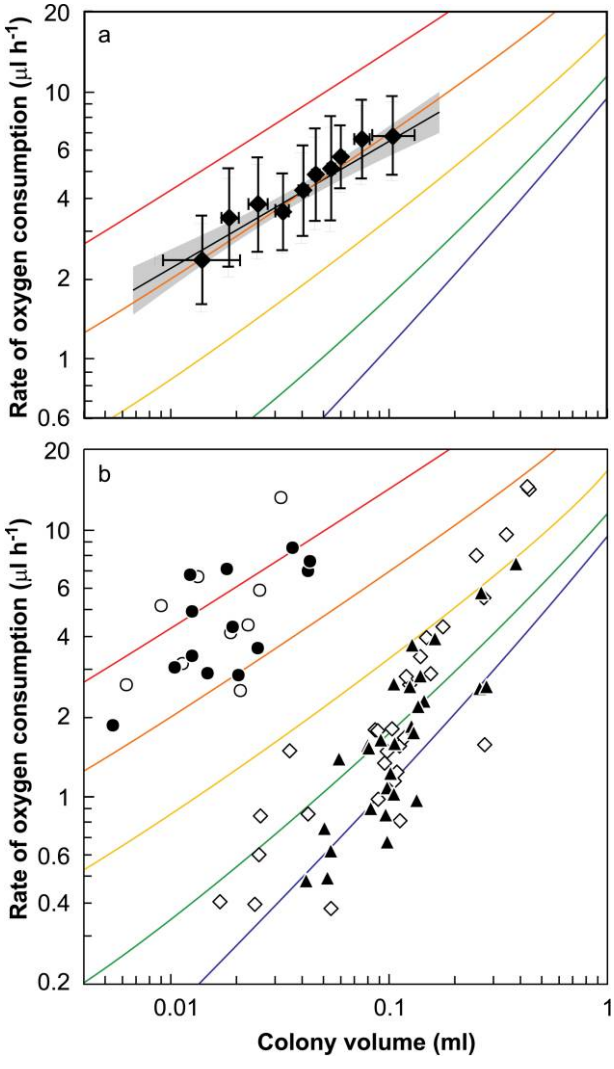

Figure 3: Measured and predicted rates of oxygen consumption for bryozoans. *a*, Rates of oxygen consumption predicted for colonies of encrusting bryozoans growing at 0.5, 2, 6, 15, and 33 cm year^{-1} (blue, green, gold, orange, and red lines, respectively). Diamonds in *a* are mean (± 1 SD) rates of oxygen consumption of *Hippoporina* colony fragments ($n = 11$–14 fragments), estimated to grow at 15 cm year^{-1} (raw data are presented in fig. 2). The black line in *a* denotes an experimentally determined scaling relationship for fragments from figure 2; the shaded area denotes the 95% confidence interval of the regression. *b*, Filled circles are measured rates of oxygen consumption of intact colonies of *Hippoporina*, which grow at 33 cm year^{-1}; open circles are edge-manipulated colonies of *Hippoporina*, estimated to grow at 24 cm year^{-1}. Diamonds are measured rates of oxygen consumption for intact colonies of Antarctic *Isoseculiflustra tenuis*; triangles are measured rates of oxygen consumption for intact colonies of Antarctic *Kymella polaris* (Peck and Barnes 2004). Ash-free dry masses of *Isoseculiflustra* and *Kymella* were converted to wet mass using species-specific dry mass to ash-free dry mass conversion factors (Peck and Barnes 2004) and assuming a water content of 84% (Stebbing 1971).

mansen et al. 2001) but shows a metabolic scaling exponent close to isometry (Hughes and Hughes 1986). In this case, the difference can be attributed to the growth form of *Electra*, which deviates increasingly from circular as size increases (Hughes and Hughes 1986). The periphery becomes expanded into lobes as the colony grows, increasing the perimeter to approximately twice that of a circle of similar area (Hughes and Hughes 1986). This has the effect of increasing the lower bound of the scaling exponent to be equal to the scaling exponent of perimeter length with colony area ($D \propto A^{\sim 0.6}$; Hughes 1986), so in this species the scaling exponent of B should be bounded closer to 1 than is the case for a circular colony. Moreover, since the lobular form of this species arises because edge zooids at the tips of the lobes grow faster than those between the lobes, mean b_e might be lower than for a circular colony. In such a situation, the scaling exponent of B should be relatively close to 1, since the extent of deviation from isometry depends on the ratio $b_e : b_c$; in nongrowing colonies, $b_e : b_c = 1$, and B scales isometrically, but in fast-growing colonies, $b_e : b_c \gg 1$, and the scaling exponent of B tends toward 0.5 (fig. 3). Small, approximately circular colony fragments of *Electra* are therefore predicted to show a metabolic scaling exponent with a lower bound of 0.5.

For colonial ascidians, the DEB model also successfully predicts the size-metabolic rate relationship under two different growth conditions (Nakaya et al. 2003, 2005). During polypide regression, all zooids within a colony of *Botrylloides simodensis* cease feeding and gradually degenerate and are replaced by small filial zooids produced by budding from parent zooids before degeneration. During this regression phase, growth of the filial zooids occurs throughout the colony, and the scaling exponent is predicted to be equal to 1 because $b_e = b_c$. The value of β during polypide regression is predicted to be higher than during normal growth, because during normal growth, only zooids at the edge of the colony grow and $b_e > b_c$. Correlational studies support this prediction: $\beta = 0.95$ and is not significantly different from 1 during polypide regression, and $\beta = 0.799$ during normal growth (Nakaya et al. 2003). Size-manipulated colonies of colonial ascidians also scale with an exponent significantly higher than that of intact colonies (Nakaya et al. 2003, 2005), as is predicted from this model if size-manipulated colonies grow more slowly than intact ones (e.g., Hart and Keough 2009).

Importantly, our DEB-based model predicts the absolute values of metabolic rate. When appropriately parameterized, the model also accurately predicts the elevation of the relationship between B and M (fig. 3). Most models require an empirically determined normalization constant, as they cannot provide a mechanistic explanation for non-size-dependent variation in B among organisms and studies. In other words, most models can predict and explain

only relative differences in metabolic rate, whereas our model explains absolute differences in metabolic rate. Overall, we demonstrate that the allometric scaling of metabolic rate in colonial animals can be explained by the DEB theory of metabolic organization, and this theory offers the appealing possibility that the observed variability in the scaling of metabolic rate in other organisms can be explained using the same principles (Kooijman 2010).

Previous studies have demonstrated that DEB theory successfully explains patterns of interspecific scaling in resting organisms (see, e.g., Kooijman 1986; fig. 8.2 of Kooijman 2010). The DEB prediction that surface-bound heating costs dominate metabolic scaling in endotherms, leading to a b value lower than for ectotherms and closer to 0.67, is also supported (White et al. 2006, 2007). The mechanisms invoked by DEB theory to explain intraspecific scaling relationships are different from those that explain interspecific scaling, and the theory also explains many intraspecific scaling relationships (e.g., Nisbet et al. 2000; van der Veer et al. 2003; Cardoso et al. 2006). DEB theory is therefore well supported as an explanation for metabolic scaling, but other theories also have considerable support.

Testing among Theories for Metabolic Scaling

A range of theories have been proposed to explain metabolic scaling, and all are successful in that they predict the scaling of metabolic rate in at least some situations. The heat dissipation limit theory (Speakman and Król 2010) explains scaling of metabolic rate in free-living endotherms but does not apply to ectotherms. The predictions for metabolic scaling of the resource-distribution model (West et al. 1997, 1999; Banavar et al. 2010) are supported to varying degrees by studies of both plants (Price et al. 2007) and animals (Savage et al. 2004; Moses et al. 2008; Kolokotrones et al. 2010), but the model has yet to adequately explain differences in the scaling exponent between endotherms and ectotherms (White et al. 2006, 2007) and between rest and activity (White and Seymour 2005; Glazier 2008, 2009*a*; White et al. 2008). The metabolic level boundaries hypothesis (Glazier 2010), on the other hand, provides a good description of variation in the scaling exponent for birds and mammals (Glazier 2008, 2009*a*), unicellular organisms (Glazier 2009*b*), and ectothermic animals (Glazier 2009*c*; Killen et al. 2010), but not all studies support the pattern (Isaac and Carbone 2010), and the hypothesis lacks a mechanistic framework (Glazier 2010). The cell size model has been supported in studies of endotherms (Vinogradov 1995; Kozłowski et al. 2003; Opazo et al. 2005), reptiles (Starostová et al. 2009), tetrapods (Vinogradov and Anatskaya 2006), and insects (Chown et al. 2007), but recent work has demonstrated that patterns of mass dependence of cell sizes in different animal groups are inconsistent with the assumptions of the model and has called for revision of the model (Kozłowski et al. 2010).

Further test of theories for metabolic scaling are therefore required to determine the generality of these theories. Such tests can be designed by examining each model to generate sets of conditions that are predicted to result in a change in the value of the scaling exponent and testing these predictions with experimental manipulations. For example, rates of growth of bryozoan colonies vary seasonally with temperature and food availability (e.g., O'Dea and Okamura 1999; Saunders and Metaxas 2009), and our model predicts that the scaling exponent of metabolic rate should vary with growth rate. Thus, if our model is correct, the scaling exponent of metabolic rate for colonies of encrusting bryozoans should vary with temperature and food availability. Such a manipulative approach represents a potentially powerful means by which theories for metabolic scaling can be tested and has the added benefit that it can be applied to organisms for which size manipulation is not possible.

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