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RESEARCH ARTICLE

Form and metabolic scaling in colonial animals

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ABSTRACT

Benthic colonial organisms exhibit a wide variation in size and shape and provide excellent model systems for testing the predictions of models that describe the scaling of metabolic rate with organism size. We tested the hypothesis that colony form will influence metabolic scaling and its derivatives by characterising metabolic and propagule production rates in three species of freshwater bryozoans that vary in morphology and module organisation and which demonstrate two- and three-dimensional growth forms. The results were evaluated with respect to predictions from two models for metabolic scaling. Isometric metabolic scaling in two-dimensional colonies supported predictions of a model based on dynamic energy budget theory (DEB) and not those of a model based on fractally branching supply networks. This metabolic isometry appears to be achieved by equivalent energy budgets of edge and central modules, in one species (*Cristatella mucedo*) via linear growth and in a second species (*Lophopus crystallinus*) by colony fission. Allometric scaling characterised colonies of a three-dimensional species (*Fredericella sultana*), also providing support for the DEB model. Isometric scaling of propagule production rates for *C. mucedo* and *F. sultana* suggests that the number of propagules produced in colonies increases in direct proportion with the number of modules within colonies. Feeding currents generated by bryozoans function in both food capture and respiration, thus linking metabolic scaling with dynamics of self-shading and resource capture. Metabolic rates fundamentally dictate organismal performance (e.g. growth, reproduction) and, as we show here, are linked with colony form. Metabolic profiles and associated variation in colony form should therefore influence the outcome of biotic interactions in habitats dominated by colonial animals and may drive patterns of macroevolution.

KEY WORDS: Bryozoa, Phylactolaemata, Coloniality, Isometry, Modularity, Respiration rate

INTRODUCTION

Constraints on physiological rates of animals derive from metabolic allometry, which describes a disproportional relationship between body size and basal metabolic rate (Kleiber, 1932; Schmidt-Nielsen, 1984). The relationship is often described by the power function $Y=aX^b$, where Y is the metabolic rate, X the body size and a the proportionality constant (Schmidt-Nielsen, 1984). Metabolic allometry occurs when the scaling coefficient $b\neq1$ and the mass-specific metabolic rate decreases with increasing body size. Allometric constraints on metabolism are widely perceived to influence phenotypic traits and to have far-reaching impacts on the evolution of biological systems (Schmidt-Nielsen, 1984; Brown et al., 2004).

The empirically measured value of b approaches 0.75 in a broad range of endothermic and ectothermic animals and many theories have been proposed to explain the dominance of such 3/4 scaling across a wide range of sizes (Kleiber, 1932; Hemmingsen, 1960; Glazier, 2010; Agutter and Tuszynski, 2011). Theories based on fractally branching supply networks predict a scaling exponent of 0.75, or alternatively 0.67 (2/3 power scaling) or 0.86 [depending on the assumptions used) (reviewed in White et al., 2011)], for two-dimensional organisms such as encrusting colonial animals (West et al., 1997; West et al., 1999; Price et al., 2007). An alternative view of metabolic scaling (based on the dynamic energy budgets model) proposes a range of scaling exponents that are predicted based on surface area, mass and energetic constraints (Maino et al., 2013; Kooijman, 1986; Kooijman, 2010).

In principle, colonial animals such as sponges, ascidians, cnidarians and bryozoans are expected to deviate from metabolic allometry because their structure entails the iteration of modules (Hughes, 2005). Isometric scaling (b=1) of colony metabolic rate and mass is thus predicted as an emergent property of modularity. This is because although the metabolic rate of a single module may scale allometrically with module size, when all modules in a colony are approximately the same size, colony metabolic rate should be a linear function of the number of modules (Sebens, 1982; Hughes, 1989). Colonial animals can therefore increase in total biomass far beyond the constraints operating on their component modules (Hughes and Hughes, 1986; Jackson and Coates, 1986). Indeed, the success of colonial lifestyles has partly been linked to the potential for indeterminate growth, enabled by iterative modular construction (Hughes, 1989). In these organisms, metabolic turnover rates and derivatives, such as rates of growth and reproduction, may therefore also converge towards isometric scaling (Hughes and Cancino, 1985; Jackson and Coates, 1986).

Isometric scaling has been demonstrated in bryozoans and ascidians with encrusting and upright two-dimensional growth (Hughes and Hughes, 1986; Nakaya et al., 2003; Peck and Barnes, 2004; Barnes and Peck, 2005). However, recently White et al. (White et al., 2011) obtained a scaling exponent of 0.50 in an encrusting colonial bryozoan (*Hippoporina indica*). They demonstrated how this result may be explained by developing a general model for colonies based on dynamic energy budget theory (White et al., 2011; Kearney and White, 2012), which we will henceforth refer to as the DEB model. This model predicts scaling exponents as a function of the collective metabolic rates of edge versus centre modules (White et al., 2011). Predicted relationships reflect the relative proportions of the position-dependent energy budgets of modules, with those at the colony edges having metabolic rates composed of both module growth and maintenance whilst non-growing modules in central regions are assumed to entail costs of maintenance alone. Hence, both growth rates and colony forms are predicted to have pervasive effects on the scaling of metabolic rate in bryozoan colonies.

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For two-dimensional colonies, the DEB model predicts that variation in growth rates will cause metabolic scaling exponents to range from 0.5 during fast growth to 1.0 during slow growth (White et al., 2011). This prediction reflects the relative energy expenditure of colony edges versus centres and is supported by comparison of metabolic scaling in slow-growing Antarctic and fast-growing Australian bryozoans (White et al., 2011). The shapes of two-dimensional colonies may also influence metabolic scaling. For instance, changes in perimeter length with colony area in non-circular colonies are expected to arise from variation in growth rates of modules within colonies and these, in turn, may result in variation in the collective metabolic rates of edge versus central modules (White et al., 2011). Notably, some two-dimensional colony growth forms may promote equivalent energy budgets of centre and edge modules, which would result in isometric metabolic scaling (White et al., 2011); however, the associated morphologies were not explicitly explored.

Both White et al. (White et al., 2011) and Kearney and White (Kearney and White, 2012) point out that distinguishing among competing explanations of metabolic rates is difficult because many models provide similar predictions. However, Kearney and White (Kearney and White, 2012) explore how explicit examination of two-dimensional encrusting organisms may enable specific testing of predictions according to the two main theories – the DEB and the West, Brown and Enquist (WBE) theories (West et al., 1997; West et al., 1999). As mentioned above, DEB theory predicts that metabolic rate will vary from 0.5 to 1.0 reflecting whether horizontal transport of metabolites is fast or slow relative to growth. In contrast, WBE theory, which is based on the geometry of the metabolic supply system, predicts that metabolic rate will be 0.67 or 0.86 (depending on the assumptions used).

Here, we tested the hypothesis that colony form will influence metabolic scaling and its derivatives by characterising metabolic and propagule production rates in freshwater bryozoans that vary in morphology and module organisation and which demonstrate two-dimensional and three-dimensional growth forms. We show that metabolic rates can differ widely with colony form and specifically evaluate our results for two-dimensional colonies with respect to predictions from the DEB versus WBE models. Our data support the DEB model and suggest that fission combined with non-circular colony growth can maintain equivalent collective energy budgets for edge and centre modules in some two-dimensional colonies. Fission may thus enable the advantages of both isometric metabolic scaling and minimising self-shading. Conversely, colonies with three-dimensional arborescent growth were characterised by allometric metabolic scaling, which is likely to be explained by self-shading or by the DEB model for isomorphs. Although our results provide support for the DEB model, they also highlight difficulties in resolving specific contributions (e.g. colony form versus growth rate) to metabolic profiles. Because metabolic rates fundamentally dictate organismal performance (e.g. growth, reproduction), our results imply that variation in colony form and module organisation will influence the outcome of biotic interactions in habitats dominated by colonial animals, such as vertical walls in subtidal temperate habitats, coral reefs and polar waters (Jackson, 1979; Peck and Barnes, 2004; Miller and Etter, 2008).

The study organisms

Freshwater bryozoans (Phylactolaemata) form colonies of identical modules (zooids) that employ a tentacular crown (the lophophore) in suspension feeding. The retractable lophophore is extended from each zooid and incurrent flow is generated by the coordinated beating of lateral and frontal cilia lining the tentacles of the lophophore (Riisgard et al., 2010). The lophophore, including tentacles and the tentacle sheath, provides a large surface area that facilitates gas exchange (Mukai et al., 1997). The importance of the lophophore for gas exchange is further suggested by the rhythmic flicking of lophophores that enhances water flow around young, non-feeding zooids in some phylactolaemate species (Wood, 1988). Thus, the arrangement and interactions of lophophores are likely to be important for colony feeding and respiration. All freshwater bryozoans produce dormant asexual propagules (statoblasts) for overwintering and colonies possess an extensive coelomic cavity in which fluid is circulated via cilia that line the peritoneal walls (Wood and Okamura, 2005).

Colony form and metabolic scaling coefficients

We examined metabolic scaling in three freshwater bryozoan species that differ in colony morphology and integration amongst modules: *Lophopus crystallinus* Pallas 1768, *Cristatella mucedo* Cuvier 1798 and *Fredericella sultana* Blumenbach 1779. *Lophopus crystallinus* and *C. mucedo* produce soft, gelatinous, two-dimensional colonies that are highly integrated with closely spaced zooids within a common large coelomic space (Fig. 1). Colonies of both species undergo fission, producing two daughter colonies that slowly creep apart. Colony growth in both species occurs by the addition of zooids parallel to the substratum; however, the resulting colony shapes are different. In the ‘caterpillar-like’ *C. mucedo* colonies, zooids are produced on either side of a central zooid-free strip (the region of degenerating zooids) along the entire length of the colony, resulting in continuous zones of lophophores deployed laterally in elongate colonies (Fig. 1A–C). Colonies grow by increasing in length but remain the same width.

Strong, overlapping feeding currents are produced by the coordinated activity of large lophophores (with some 70+ tentacles) (Wood and Okamura, 2005) running along each side of elongate *C. mucedo* colonies (B.O., personal observation). Excurrents are directed below the closely spaced lophophores (from the colony margin towards the centre) and are then voided at high velocity through the narrow, lophophore-free central strip away from the colony surface (B.O., personal observation; Fig. 1C) as a result of the high incurrent:excurrent area ratio (Lidgard, 1981). This arrangement of lophophores allows colonies to avoid reprocessing excurrent water and to obtain resources from regions in the water column inaccessible to more widely spaced lophophores that act individually (Eckman and Okamura, 1998). These two interdependent phenomena (avoidance of reprocessing and overlapping feeding currents) will minimise self-shading or interference (Grünbaum, 1995; Eckman and Okamura, 1998; Kim and Lasker, 1998). Because *C. mucedo* colonies retain the same width, total colony length is expected to be directly proportional to colony mass and, unlike the circular colonies that are the main focus of the DEB model of White et al. (White et al., 2011), the relative proportions of zooids involved in growth and maintenance do not change as colony size increases. *Cristatella mucedo* metabolic rate is therefore expected to scale with colony size isometrically (b=1), contrasting with the value of 0.5 that characterised the circular colonies of *Hippoporina* measured by White et al. (White et al., 2011).

Zooids in *L. crystallinus* are budded in a frontal–lateral direction to produce closely spaced lophophores in a fan-like array of up to 40 zooids (Wood and Okamura, 2005) (Fig. 1D–F). As in *C. mucedo*, overlapping feeding currents are produced amongst the closely spaced, distally orientated, large lophophores (with some 70

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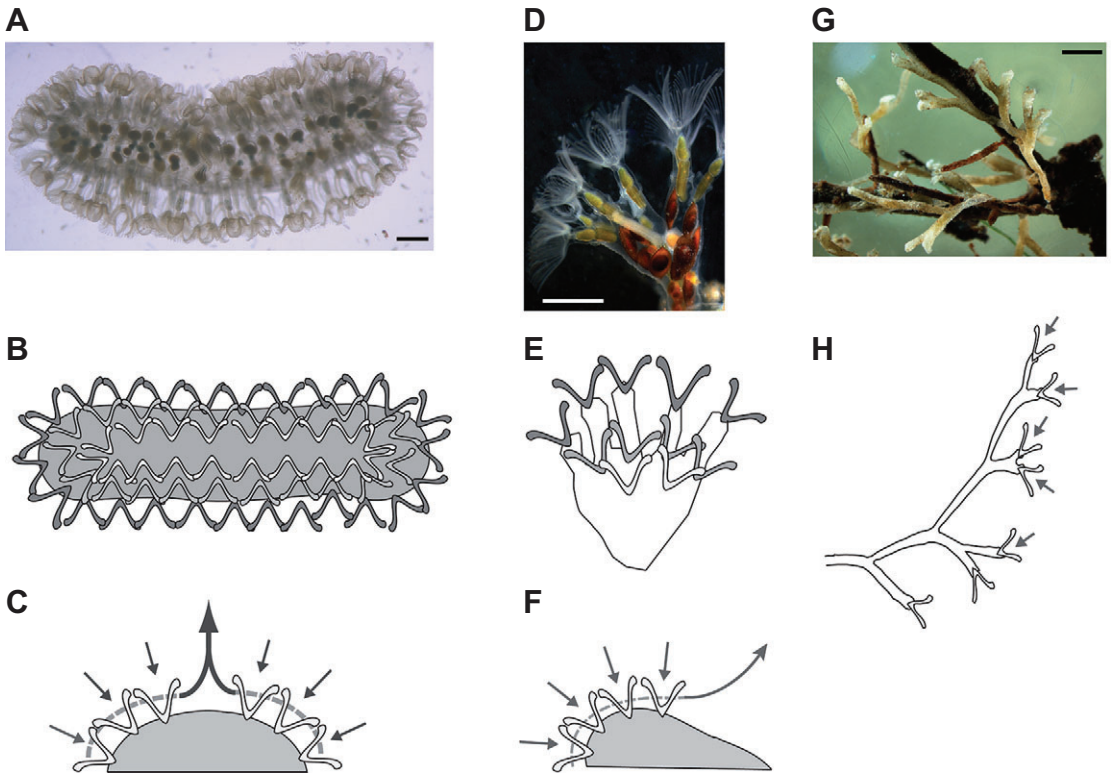

Fig. 1. Typical colony morphologies of the elongate *Cristatella mucedo* (A–C), the fan-shaped *Lophopus crystallinus* (D–F) and the branching, erect *Fredericella sultana* (G–H). Schematic representations of colony morphologies show locations of lophophores (V-shapes) extended from colony surfaces (grey shading) in each of the three species (B,E,H). Feeding currents (small arrows in C,F,H) produced by cilia lining the lophophores are directed centrally towards the mouth at the base of the lophophores. Excurrents are directed below arrays of closely spaced lophophores and escape as a jet away from colony surfaces (reducing the potential for refiltration) in *C. mucedo* (C) and *L. crystallinus* (F) as shown by the large arrow. Note that the widely spaced lophophores of *F. sultana* preclude the formation of an excurrent jet, thus increasing the potential for refiltration. Scale bar represents 1 mm.

tentacles) (Mundy, 1980) and excurrents are directed towards the lophophore-free, proximal region of the colony (Fig. 1F). The budding zone occurs at the leading edge of the fan-shaped colonies, with older degenerate zooids present in the distal, trailing region (Fig. 1D–F). The morphology in this case is somewhat similar to ‘a wedge’ of a circular, encrusting colony. The DEB model would therefore predict scaling exponents from 0.5 to 1.00 (White et al., 2011), depending on, for example, growth rate, colony form and the frequency of colony fission.

In contrast, *Fredericella sultana* Blumenbach 1779 produces three-dimensional, arborescent colonies composed of tubular, occasionally bifurcating branches that ramify both across and above the substratum (Fig. 1G,H). Relatively widely spaced zooids with small lophophores (some 16–27 tentacles) (Wood and Okamura, 2005) occur periodically along the branches. Each zooid is capable of budding two new zooids and as colonies increase in size, the growing zooids mostly occupy the space on the surface of the bush-like colony. As the shape of this bush is not expected to change during growth, the DEB model treats arborescent colonies as isomorphs and 2/3 scaling is predicted (b=0.67) (Kooijman, 2010).

RESULTS

Isometric scaling characterised colonies of *C. mucedo* and *L. crystallinus*, while allometric scaling characterised colonies of the branching bryozoan *F. sultana* (Fig. 2A–C). The metabolic rate of *F. sultana* had a scaling coefficient of 0.61, similar to the surface area:volume scaling exponent (0.67), indicating allometric constraints on metabolic rate in this species. The inferred exponent of 0.61 was significantly different from the hypothesised exponent of 1.00 (r=–0.63, P<0.001), and from 0.86 (r=–0.48, P=0.0002) but not from 0.67 (r=–0.15, P=0.272). *Cristatella mucedo* had a coefficient of 1.12, which was not significantly different from 1.00 (r=0.19, d.f.=46, P=0.190), but differed significantly from 0.86 (r=0.40, d.f.=46, P<0.002) and from 0.67 (r=0.59, d.f.=46, P<0.0001). In *L. crystallinus*, the scaling coefficient of 1.19 was not significantly different from 1.00 (r=0.21, P=0.07), but differed significantly from 0.86 (r=0.50, P<0.0002) and from 0.67 (r=0.60, P<0.0001).

Cristatella mucedo colonies increased in length without significant changes in colony width, as, across a colony length range of 8–48 mm, the colony width varied only between 6 and 8 mm (the shortest colony being 8 mm wide) (P=0.970; see supplementary material Fig. S1).

The production of asexual resting stages, statoblasts, in both *C. mucedo* and mature *F. sultana* scaled isometrically relative to colony size (replication was too low to include *L. crystallinus*) (Fig. 3). In *F. sultana*, statoblast production was initiated in the majority of colonies that were larger than five zooids so small colonies were excluded from analysis. The scaling coefficient for statoblast production in mature *F. sultana* colonies (>5 zooids in size) was 1.08 (confidence interval CI 0.9–1.22), which was not significantly different from 1 (P=0.222) and was significantly different from 0.67 and 0.75 (P<0.0001 in both cases). We note that the statoblast counts

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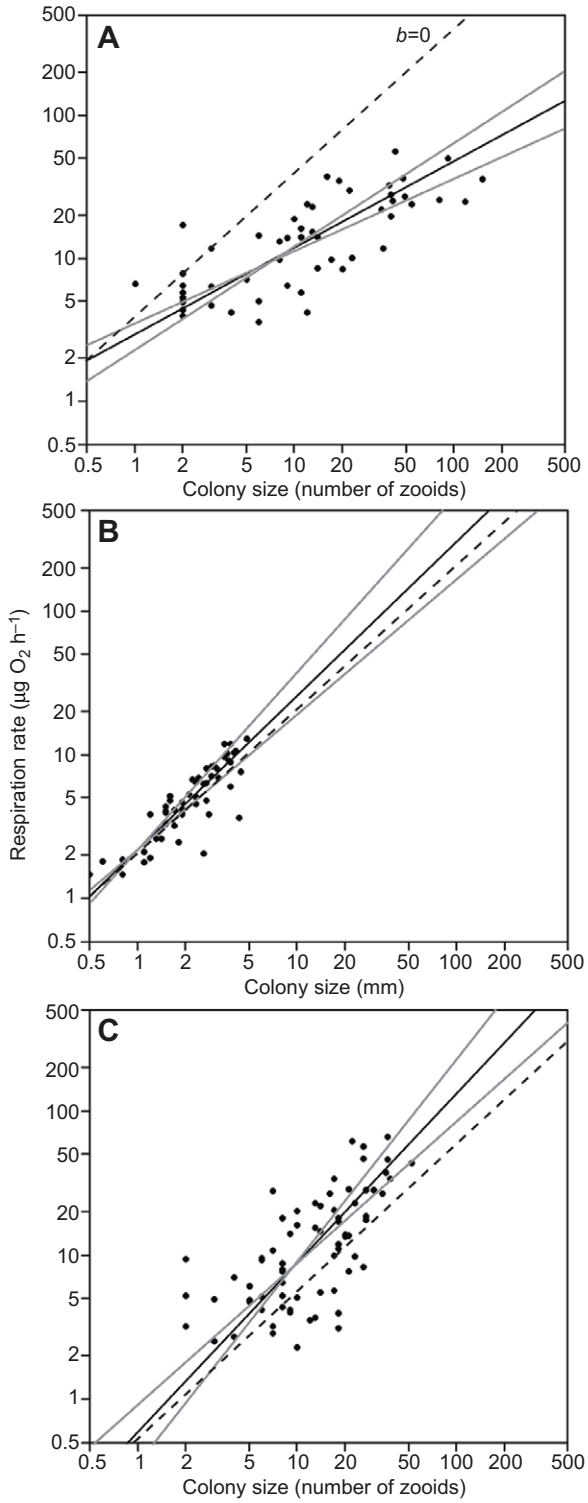

Fig. 2. Relationship between respiration rate and colony size. (A) *Fredericella sultana* (size measured by number of zooids); (B) *C. mucedo* (size measured as colony length in mm); and (C) *L. crystallinus* (size measured as number of zooids). Grey lines indicate the slopes of the 95% confidence limits for *b* and the dashed line shows the hypothesised slope *b*=1 with the same intercept as the experimentally derived slope.

for *F. sultana* in some cases included statoblasts in regions of the colony where zooids had degenerated. However, as the number of degenerate zooids increased as a constant proportion to the number of live zooids, the slope of the regression should not be biased. Furthermore, a regression of total colony size (live+dead zooids) against total number of statoblasts gave a similar result (b=1.13, CI 1.02–1.27). In *C. mucedo*, the coefficient for statoblast production relative to colony length was 1.01, which was significantly different from both 0.67 and 0.75 (P<0.001 in both cases). There was no clear size dependency of statoblast production, which probably reflects the fact that mature *C. mucedo* colonies divide by fission.

DISCUSSION

Modular organisation, colony form and metabolic scaling

As predicted by the DEB model (White et al., 2011) for two-dimensional organisms, colony size scaled isometrically with respiration rate in both *C. mucedo* and *L. crystallinus*. The DEB model predicts this result under assumptions of slow colony growth or if colonies maintain equivalent and constant energy budgets for ‘edge’ and ‘central’ (i.e. growing and non-growing) modules. We suggest the latter applies in both cases but is achieved in different ways. In *C. mucedo*, new zooids are budded in two opposing directions along the length of the colony, resulting in a central strip of degenerating zooids deriving from the opposing lateral budding zones. The colonies thus retain standard energy budgets by increasing colony length whilst retaining approximately standard width. The metabolic isometry afforded by the elongate shape of *C. mucedo* colonies may allow the development of a range of colony sizes, which appear to reflect the nature of the substratum. Thus, very elongate colonies occur on narrow, straight stems of macrophytes, while shorter colonies are found on more expansive surfaces (e.g. lily pads), where micropredators are often abundant and across which daughter colonies can move in multiple directions (B.O., personal observation). *Cristatella mucedo* colonies can attain large sizes (in the field colonies can grow to ~20 cm) and coelomic fluid is circulated via ciliary beating throughout the continuous body cavity (Fig. 1). Metabolites are thus distributed and shared effectively across the whole colony, which may facilitate fast growth rates in small colonies. As colonies increase in length, the budding regions directly involved in colony elongation at the ends of the colony presumably become independent of colony size and colony mass-specific growth rates decline. Such limitations in growth rate may be involved in the onset of colony fission and directly affect the average size that colonies develop to on a given habitat patch.

The fan-shaped colonies of *L. crystallinus* result from zooidal budding orientated essentially in a single direction resulting in a distal zone of active zooids and a proximal region of degenerating zooids. Unidirectional budding in *L. crystallinus* would be expected to eventually lead to metabolic constraints as fan-like colonies increase in size. The key process explaining the observed isometry for *L. crystallinus* is therefore likely to be the regular colony fission that is characteristic of the species and the subsequent movement (to avoid shading) of daughter colonies. Fission thus ensures that *L. crystallinus* colonies do not exceed sizes that produce inequalities in the energy budgets of edge and central modules.

Slow growth is unlikely to explain isometry for either species. The *L. crystallinus* colonies used for respiration measurements were maintained for several months in benign laboratory culture systems that have been demonstrated to promote growth in *F. sultana* (Hartikainen et al., 2009; Tops et al., 2009). Although we did not monitor growth rates of the *L. crystallinus* colonies in culture, they are expected to be similarly stimulated. Slow growth is also unlikely to have characterised the *C. mucedo* colonies as

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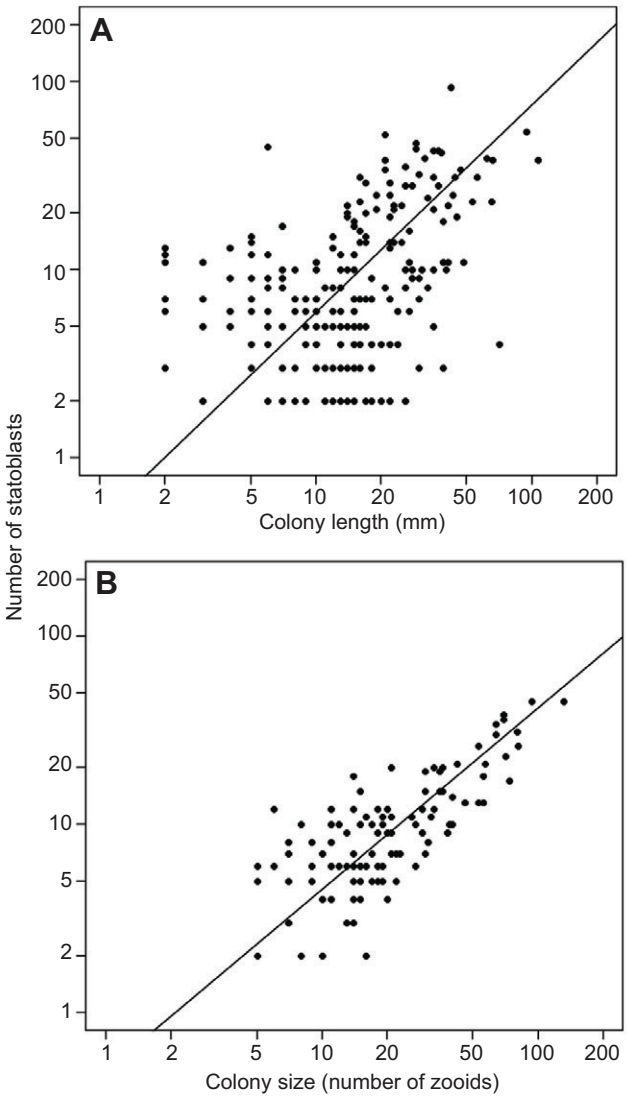

Fig. 3. Relationship between number of statoblasts and colony size. (A) *Fredericella sultana* (colony size measured as number of zooids); and (B) *C. mucedo* (colony size measured as length in mm).

these were collected at a time when colonies undergo explosive growth in the field. Furthermore, the colonies of *L. crystallinus* and *C. mucedo* were generally not producing statoblasts, indicating that metabolites were directed to active growth, fission and movement, rather than production of resistant resting stages.

Our results for *F. sultana* also support predictions of the DEB model, in this case for metabolic allometry in arborescent isomorphs. The scaling exponent was not significantly different from 2/3 power scaling (b=0.61). Our collective results for both two- and three-dimensional colonies therefore provide evidence that the DEB model can explain general constraints in metabolic scaling at the colony level. They also suggest how life history (e.g. colony fission) can influence metabolic scaling and demonstrate how drivers of metabolic scaling may be misconstrued. Thus, in two-dimensional colonies, scaling coefficients can be equally explained by slow growth or when colonies maintain equivalent and constant energy budgets for 'edge' and 'central' modules. Determining the relative contributions of these factors may sometimes be problematic.

Self-shading, colony support and metabolic scaling

A number of previous studies have stressed the importance of self-shading in constraining the acquisition and transport of energy and materials in colonial animals (Kim and Lasker, 1998). For instance, self-shading has been demonstrated in a wide range of colonial organisms when modules in the centre of the colony may not feed at all or receive fewer food items than peripheral modules (Sebens, 1982; Okamura, 1984; Okamura, 1985; Kim and Lasker, 1998). Modules located in 'downstream' positions may also receive fewer food items and spend more energy by processing water already depleted of food items by the 'upstream' colonies (Okamura, 1984; Okamura, 1985; McFadden, 1986; Grünbaum, 1995; Kim and Lasker, 1998). These previous studies have demonstrated the importance of self-shading for resource capture in large colonies and it thus seems plausible that the effect of self-shading on resource capture may drive some of the scaling patterns observed in the present study.

Adaptations to compensate for self-shading in plants include maximising light use efficiency via changes in the distribution of leaves on the surface versus within the canopy and changes in canopy structure with size (Duursma et al., 2010). *Cristatella mucedo* and *L. crystallinus* may similarly change the size of the 'lophophore canopy' to minimise self-shading by colony fission dependent on resource availability. Fission may maximise resource capture rates if it avoids diminishing returns beyond a critical size due to self-shading to produce daughter colonies whose sizes confer the benefit of feeding current integration and/or avoidance of metabolic constraints. Empirical support for this kind of scenario was gained in a study of soft corals that undergo fission (McFadden, 1986). Small colonies were demonstrated to be more efficient at feeding. Furthermore, colonies tended towards equivalent small sizes and maintained regular intercolonial spacing, thereby achieving maximal feeding rates. We have observed such regular spacing between colonies of equivalent sizes in dense aggregations of *L. crystallinus* (H.H., personal observation) and *C. mucedo* (B.O., personal observation).

A corollary of maintaining intercolonial spacing to avoid self-shading is that metabolic isometry may also characterise *L. crystallinus* and *C. mucedo* aggregations. The energetic equivalence rule (EER) states that the population metabolic rate is a product of the number of individuals and the average metabolic rate of the individuals (Damuth, 1981). Thus, the population metabolic rate is independent of body size if the colony metabolic rate scales as size to the power of b and population density scales as size to the power of $-b$. However, EER predictions have rarely been tested and a recent study found that eusocial insect colony metabolism was dependent on the average body size of the individuals (DeLong, 2011). Freshwater bryozoans represent a useful model system for examining whether metabolic isometry at the colony level is also exhibited in colony aggregations because aggregations with different intercolonial spacings and average colony sizes can be experimentally created. Assuming EER, it would be predicted that at prevailing conditions of, for example, flow regime, food concentration, dissolved oxygen levels and density, fission may optimise colony size for escape from resource depletion, competition and self-shading. We would therefore expect to see variation in colony size in different habitats – an observation that is apparent for *C. mucedo*, which exhibits substantial spatial and temporal variation in mean colony size (B.O., unpublished data).

A common developmental feature that can impact metabolic scaling is that as organisms increase in size relatively more tissue is invested in metabolically inactive structures, such as stems or supportive skeletons (Niklas et al., 2007; Koontz et al., 2009). This

proportional increase in non-respiring biomass results in metabolic scaling exponents of <1, even when isometry would be expected based on colonial organisation (Koontz et al., 2009). Such investment in support, however, cannot explain the allometry observed in *F. sultana*. Zooids of *F. sultana* are relatively invariant in size (Toriumi, 1951; Hartikainen et al., 2013) and no additional 'spacer zooids' are required to achieve larger colony sizes. The observed allometry therefore is in keeping with predictions of the DEB model for isomorphs. It could also be imposed by self-shading resulting from three-dimensional colony growth and lack of feeding current integration that could potentially compensate for self-shading (processes that explain the surface area:volume scaling exponent predicted by the DEB model). Thus, both reprocessing of excurrents by zooids within the colony and flow diversion around the colony (Chamberlain and Graus, 1975; McKinney et al., 1986; Grünbaum, 1995) should contribute to self-shading.

Energetic constraints and scaling of propagule production

Our study and that of Barnes and Peck (Barnes and Peck, 2005) on Antarctic bryozoans support the prediction that size increases for colonies with three-dimensional morphologies may be more costly than for many colonies with two-dimensional morphologies. However, we detected no allometric constraints in the production of asexual propagules, which increased isometrically with colony size in both colony forms. This contrasts with the marked allometry in metabolic rates of arborescent *F. sultana* colonies but is consistent with isometry in the metabolic rates of two-dimensional *C. mucedo* colonies. It therefore appears that the reproductive output of many small colonies will be equivalent to the reproductive output of a single large colony of the same total number of zooids in the two species (provided there are >5 zooids in the case of *F. sultana*). This result may be explained by a physiological limitation on the number of statoblasts produced by an individual zooid. For instance, *F. sultana* produces a maximum of two statoblasts per zooidand thus the number of statoblasts is proportional to the number of zooids (i.e. colony size). Notably, the DEB model predicts that growth rates should scale nearly isometrically with colony size in branching colonies and our empirical data from reproductive output support this prediction. In *C. mucedo*, isometric scaling of statoblast production is expected to be unaffected by colony fission. This is because the smaller daughter colonies should contain equal proportions of statoblasts, reflecting the fact that the budding zones of *C. mucedo* colonies extend continuously along the entire colony length. The zooid populations of daughter colonies will therefore be composed of equal age distributions and should thus contain equivalent numbers of, for example, incipient, developing and mature statoblasts; this suggests a similar mechanism to *F. sultana*, with a physiological limit to the number of statoblasts produced by a zooid.

Metabolic scaling, ecology and macroevolution

The overall observed variation in metabolic scaling coefficients for two- and three-dimensional colonial organisms (e.g. Barnes and Peck, 2005; White et al., 2011) (this study) implies that ecological models based on commonality of metabolic scaling relationships may lead to biased predictions. Yet, interpreting how colony form drives dynamics observed at the population and community levels remains poorly investigated and requires special attention despite a long-standing appreciation of the importance of form and function in colonial animals (Jackson, 1979).

The energetic constraints imposed by the varying metabolic scaling relationships may play a significant role in mediating competition for space (Jackson, 1979) and for oxygen (Ferguson et al., 2013). For example, the increasing per-module energy demand in larger arborescent colonies may have contributed to the decline in the relative abundance of bryozoans with three-dimensional, upright growth after the Paleozoic era (McKinney and Jackson, 1989). Three-dimensional forms may initially have been selected to effect escape from substratum-related biotic interactions, but escalation in the effectiveness of partial predation on colonies in the Mesozoic era may have subsequently selected for cryptic, encrusting forms (McKinney and Jackson, 1989). Energetic constraints may have helped to drive these outcomes by limiting regeneration capacities of three-dimensional forms. Energetic constraints may also have contributed to the extinction of all upright, three-dimensional forms of *Metrarabdotos* spp. in the Caribbean following the closure of the Isthmus of Panama, despite the persistence of encrusting *Metrarabdotos* spp. in the region (Cheetham and Jackson, 1992). The closure of the isthmus entailed a dramatic decline in productivity in the Caribbean (O'Dea et al., 2007) and thus the costs of producing and maintaining upright, three-dimensional growth may have been prohibitive. However, the demonstration that b=0.5 in *Hippoporina* (White et al., 2011) indicates that two-dimensional, encrusting bryozoans may also be subject to allometric constraints. The evolutionary drivers selecting for such forms is an intriguing area for future research.

Conclusions

Freshwater bryozoans present an excellent model system for characterising the metabolic scaling coefficients associated with different colony sizes and shapes and for testing predictions of metabolic theory. Their particular advantage is that they produce only feeding zooids and therefore avoid the biases introduced by the presence of nutritionally dependent, polymorphic modules (e.g. brooding ovicells, defensive avicularia and vibracula) that are present in most marine bryozoan colonies, including those that have been the focus of previous metabolic rate studies (Peck and Barnes, 2004; Barnes and Peck, 2005; White et al., 2011). As the density and presence of these nutritionally dependent modules is environmentally induced, their contributions to growing and consuming biomass within colonies are likely to be complex and are currently ignored in the DEB model of White et al. (White et al., 2011). Future studies that examine metabolic scaling in marine bryozoans in relation to predictions of the DEB model will need to address or experimentally control for these added intricacies of consuming biomass by non-feeding colony components. Further biases may be introduced depending on the size and developmental stage of the experimental colonies.

Our results demonstrate that colonial animals are characterised by a range of metabolic scaling relationships that are in accord with predictions from the DEB model for the elongate *C. mucedo* (which represents a V1 morph) (Kooijman, 2010), for the arborescent *F. sultana* (an isomorph) (Kooijman, 2010) and for the fan-shaped *L. crystallinus* (as a result of fission). We thus identify organismal traits that are likely to contribute to the wide range of metabolic scaling coefficients observed in bryozoans and suggest how such traits may dictate patterns of distribution and abundance over space and time. Important questions for future studies include understanding the trade-offs that favour arborescent forms despite the metabolic cost of allometry and self-shading, the trade-offs associated with the evolution of metabolically dependent polymorphs within colonies and how colony morphology determines the metabolic biomass of colonial consumers and their roles in benthic food webs.

MATERIALS AND METHODS

Individual colonies of *F. sultana* and *L. crystallinus* were maintained in laboratory culture systems (Hartikainen et al., 2009) for a minimum of 2 weeks prior to measurements. *Cristatella mucedo* colonies were collected from the field because they are less readily maintained in culture systems. The colonies were allowed to attach to large acetate sheets in the laboratory and individual colonies were then targeted for respiration measurement by cutting the sheet around the colonies. The *C. mucedo* colonies were acclimatised to 20°C for 24 h before measurements were taken. During this time colonies were maintained in filtered aquarium water in order to minimise variation between individuals that may have been feeding on different food sources in the field.

Colony size (the number of live zooids in *F. sultana* and *L. crystallinus*, colony length and width in *C. mucedo*) and the number of statoblasts were recorded prior to measurement. The experimental colonies were gently cleaned with forceps and the plastic sheet or disc around the bryozoan was wiped clean. The colonies were placed in filtered aquarium water in Petri dishes and allowed to recover from handling for 45 min. Oxygen consumption of each colony was measured using an optode system (Fibox 3, PreSens, Regensburg, Germany). Respirometry of *F. sultana* (N=53, size range 1–150 zooids) and *L. crystallinus* (N=71, size range 2–52 zooids) took place in Petri dishes (5.5 cm wide, 1.5 cm high), filled with filtered aquarium water at the same temperature as the laboratory culture systems (20°C). The lid of the vessel was sealed with petroleum jelly and submerged in an insulated waterbath. Colonies were allowed to recover for a further 15 min before oxygen concentration was automatically recorded every 10 s for 3 min. This was repeated four times at approximately every 18 min for each individually sealed colony. Water within the experimental vessels was not stirred during the measurements to avoid disturbance to the bryozoans (retraction of lophophores would interfere with respiration measurements). For *C. mucedo* (N=53, size range 0.4–4.8 mm), we used a 10-channel Fibox setup (OXY-4/10 Mini, PreSens), and each colony was measured continuously for up to 60 min in a stoppered measurement vessel containing 50 ml of filtered aquarium water. Conditions for measurements were otherwise identical in all species and a control vessel with no colony was included with every batch of measurements taken.

Oxygen consumption rates per colony were calculated by fitting an ordinary least squares linear regression to the oxygen concentration within the experimental vessel as a function of time (μg O_2 h^{-1}). Only those measurements where R^2 was >80% were accepted for further analysis and the rates were corrected for any background O_2 consumption in the control vessel measured at the same time as the experimental colonies. The slopes of log–log linear functions of size-specific scaling of respiration rate and reproductive effort were calculated using standardised major axes (SMA) regressions within the smatr package in R (version 12.1.1). The slopes of the 95% confidence limits were plotted using package lmodel2. SMA regressions were chosen as the aim was to describe the relationship (b) between the traits of interest, rather than the predicted values (a) or strength of the relationship (R^2) (Warton et al., 2012). The empirically derived slopes of the metabolic scaling relationships were compared with 0.67 or 0.86 and with predictions of DEB models for each bryozoan species tested.

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Competing interests

The authors declare no competing financial interests.

Author contributions

All authors contributed to the conception, design, execution and interpretation of the findings.

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Supplementary material

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