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SHORT COMMUNICATION

Conspecific chemical cues drive density-dependent metabolic suppression independently of resource intake

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ABSTRACT

Within species, individuals of the same size can vary substantially in their metabolic rate. One source of variation in metabolism is conspecific density – individuals in denser populations may have lower metabolism than those in sparser populations. However, the mechanisms through which conspecifics drive metabolic suppression remain unclear. Although food competition is a potential driver, other density-mediated factors could act independently or in combination to drive metabolic suppression, but these drivers have rarely been investigated. We used sessile marine invertebrates to test how food availability interacts with oxygen availability, water flow and chemical cues to affect metabolism. We show that conspecific chemical cues induce metabolic suppression independently of food and this metabolic reduction is associated with the downregulation of physiological processes rather than feeding activity. Conspecific cues should be considered when predicting metabolic variation and competitive outcomes as they are an important, but underexplored, source of variation in metabolic traits.

KEY WORDS: Metabolic variation, Population density dependence, Metabolic reduction, Competition, Energy flux

INTRODUCTION

Metabolic rate is one of the most measured physiological traits as it is linked to the rate at which organisms acquire resources and transform them into energy to sustain biological structures and processes (Brown et al., 2004; Kearney and White, 2012). Understanding sources of variation in metabolism is necessary to estimate energy flows, from the level of individual organisms through to whole ecosystems (Humphries and McCann, 2014; Marquet et al., 2004). Although body size is a good predictor of metabolism across species, individuals of the same species vary substantially in metabolism, even after accounting for size (Burton et al., 2011; Norin and Metcalfe, 2019). Conspecific interactions could explain some of this residual variation (Brown et al., 2004; DeLong et al., 2014; Humphries and McCann, 2014), but the specific drivers through which conspecifics suppress metabolism remain largely unknown.

Individuals in denser populations often have lower metabolic rates than individuals in sparser populations (DeLong et al., 2014; Ghedini et al., 2017; Malerba et al., 2017), but it is unclear what drives this response, which is not consistently observed (Sereni and Einum, 2015; Yashchenko et al., 2016). Food is an obvious factor: at high densities, individuals experience increased intraspecific competition and their food consumption decreases (Amundsen et al., 2007; DeLong et al., 2014). Because food consumption increases metabolic rate, an effect known as specific dynamic action (SDA; Secor, 2009), reductions in food intake also reduce metabolism (Schuster et al., 2019). Such a response might be adaptive – by reducing their food and energy requirements, organisms might be better able to survive and conserve energy reserves when competition is high (McCue, 2010; Auer et al., 2015). Therefore, understanding the drivers of density-dependent metabolism is important not only to explain metabolic variation among conspecifics, but also to predict its consequences for population dynamics (Marquet et al., 2004). But is food the only driver of metabolic suppression?

Metabolic suppression can occur even when organisms do not experience reductions in food availability (DeLong et al., 2014), albeit this response is not consistently observed (Yashchenko et al., 2016). Therefore, it remains unclear whether density-dependent metabolism is only driven by food availability or also by other conspecific changes in the environment that might interact with resource supply (Okamura, 1984; Killen et al., 2012; Thompson et al., 2015). For example, conspecifics can alter levels of oxygen availability (Yashchenko et al., 2016), access to sunlight or wind patterns (Broz et al., 2010), or the biotic environment by releasing chemical cues (Nadler et al., 2016; Pereira et al., 2017). Therefore, metabolic suppression in response to conspecifics might be a more complex response than currently thought.

In the marine environment, sessile organisms living at high densities can alter two abiotic conditions that are key for survival: oxygen availability and water flow (Lagos et al., 2015). Because the uptake of food and oxygen in sessile organisms depends on the replenishment of the boundary layer, dense populations can disrupt water flow, altering access to both food and oxygen (Okamura, 1984; Ferguson et al., 2013). If these abiotic changes signal intense competition, organisms may respond to low flow or oxygen environments by suppressing their metabolism (Kim and Lasker, 1997; DeLong et al., 2014).

Conspecifics might also release cues that trigger changes in metabolic rates. For instance, allelopathic chemicals that reduce metabolism in competitor species (Poulson-Ellestad et al., 2014) might have similar effects on conspecifics. Conversely, conspecific cues might have beneficial effects, for instance allowing group-living organisms to reduce metabolism (i.e. 'calming effect', Nadler et al., 2016) or signal predation risk (Gibson and Mathis, 2006; Pereira et al., 2017). Metabolism is therefore highly plastic to conspecifics (Norin and Metcalfe, 2019) but it remains unclear to what extent metabolic changes are driven by food availability and its interactions with other conspecific effects on the environment.

In a series of experiments, we disentangled the effects of food availability from other possible drivers of metabolic suppression. Specifically, we tested whether metabolic rates in a sessile marine invertebrate were reduced in response to food availability in

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Received 9 March 2020; Accepted 17 July 2020

Journal of Experimental Biology (2020) 225, jeb224824. doi:10.1242/jeb.224824

combination with abiotic (reduced oxygen availability and water flow) and biotic drivers (conspecific chemical cues). We then explored the role of conspecific chemical cues independently of food. Finally, we tested whether metabolic suppression was associated with changes in foraging activity. We used *Bugula neritina* as a model system, a colonial bryozoan found in medium to low flow coastal environments (Keough, 1989; Lagos et al., 2017). We chose this species because individual colonies display density-dependent metabolic rates in response to conspecific colonies (i.e. among-colony competition; Ghedini et al., 2017) and can detect conspecifics more generally (Thompson et al., 2015).

MATERIALS AND METHODS

Study species

Colonies of *Bugula neritina* (Linnaeus 1758) were collected from Royal Brighton Marina, Victoria, Australia, during autumn and winter 2019. Colonies were transported to the laboratory, where they were kept unfed in complete darkness for 2 days in aerated, filtered (0.2 µm filter) seawater. Colonies were then exposed to a bright light for 30–60 min to induce larval spawning, following methods of Marshall et al. (2003). Larvae were settled onto roughened PVC plates (5×5 cm, 5 settlers per plate). Plates were deployed at the original collection site the day after settlement, attached among four PVC panels hanging from the pier and submerged facing downwards at 1–2 m in depth. Four weeks after deployment, excess settlers were culled so only one focal colony remained on each plate (N=105 total colonies) and plates were cleared of all other organisms every 2 weeks. Colonies were grown in the field for 11 weeks before being transported back to the laboratory for metabolic measurements.

Metabolic rates

Oxygen consumption was measured in air-tight, acrylic water baths (16×13×5 cm). Each bath was connected to a pump (Kamoer Dosing Pump, model KSP-F, Shanghai, China) that recirculated seawater in a closed-loop system, and the rate of oxygen consumption was measured with a flow-through sensor connected to a fibre-optic oxygen meter (Pyro Science, Aachen, NRW, Germany). Oxygen consumption was measured as change in percent air saturation over 2.5 h at 1-min intervals (Lagos et al., 2017; Ghedini et al., 2017). The change in oxygen levels was calculated with linear regressions using the LoLinR package (Olito et al., 2017). Metabolism was measured at a constant room temperature of 19°C. The average change in oxygen concentration in 'blank' water baths, containing seawater and a plate with no colonies, was used to account for background bacterial respiration. The oxygen consumption of each colony was used as a proxy for metabolic rate and was calculated in millilitres per hour (Alton et al., 2007) as:

$$\dot{V}_{O_2} = -1[(m_b - m_c)/100] \times V \times \beta_{O_2}, \quad (1)$$

where m_b is the rate of change of oxygen saturation in water baths with colonies (% air saturation), m_c is the rate of change in oxygen in the blank water baths, V is the volume of water in each bath (litres) and β_{O_2} is the oxygen capacitance of air-saturated water at 19°C (=5.31 ml l^{-1}; Cameron, 1986). After measuring metabolic rates, all colonies were dried for 11–14 h in a drying oven at 60°C before measuring their dry mass on an electronic scale (±0.01 g).

Experiment 1 – food and abiotic factors: oxygen depletion and water flow

We tested the metabolic response of colonies to food (fed versus starved) in combination with two abiotic factors (oxygen depletion and disrupted water flow), and a control of unmanipulated seawater. Each day we tested colonies from one panel. Half of the colonies were assigned to the fed treatment and the other half to the starved treatment. Within each food treatment, colonies were randomly assigned to one of three water treatments: oxygen-depleted seawater (n=1), disrupted water flow (n=1) or a control group (n=3). This design was repeated over four consecutive days (runs), so in total 12 fed and 12 starved colonies were assigned to the control water treatment, and four fed and four starved colonies were each assigned to the remaining water treatments (N=40).

Colonies in the starved treatment were kept overnight in individual 500 ml plastic containers, filled with 400 ml of filtered seawater with no food. Those in the fed treatment were kept under the same conditions, but supplemented with excess amounts of the microalga *Dunaliella tertiolecta* (20,000 cells ml^{-1}) during the night and again 3 h before their metabolic rates were measured. All colonies were rinsed in filtered seawater to remove any algae before measuring metabolism.

Each colony was acclimated to its assigned water treatment for 30 min before measuring metabolism. For the oxygen-depletion treatment, we used seawater with oxygen levels of 60–65% air saturation, well above the oxygen concentration recorded for this species, which is ∼20% (Lagos et al., 2017). Oxygen levels were reduced by bubbling seawater with nitrogen gas (Fan et al., 2014). The disrupted flow treatment, which simulated changes in water flow owing to the physical presence of conspecifics, was created by attaching four plastic mimics around a central focal colony (Thompson et al., 2015). Colonies in the control group were placed in water baths filled with untreated filtered seawater. Four additional water baths were used as blanks (i.e. without colonies) to account for background microbial respiration, two filled with normal seawater and two with oxygen-depleted seawater.

Experiment 2 – food and conspecific chemical cues

Fed and starved *B. neritina* colonies were grown and exposed to the same food treatments as in experiment 1, but were exposed either to conspecific chemical cues (n=8) or to a control treatment (n=24) over four experimental runs. Colonies assigned to the chemical cue treatment were exposed to seawater that had been conditioned with *B. neritina* colonies by placing nine colonies (>7 bifurcations) in a 10 litre cooler box filled with bubbled seawater overnight, then filtered with a 0.2 µm filter to remove any debris. Colonies in the control group were placed in water baths filled with normal filtered seawater that had not been exposed to *B. neritina* colonies.

Experiment 3 – the role of conspecific chemical cues

Given the indication for a reduction in metabolism in colonies exposed to chemical cues (see Results), we tested the effects of conspecific cues in starved colonies alone to increase the replication of the study. Metabolic measurements were conducted over nine runs during which a total of 19 colonies were assigned to the chemical cue treatment and 27 to the control treatment. All methods were as above but without the food treatment, so all colonies were starved overnight before metabolic measurements.

Experiment 4 – is metabolic suppression linked to reduced feeding behaviour?

Following the same procedures as described above, we grew *B. neritina* colonies in the field for 3 weeks. Larvae were deployed on acetate sheets attached to a PVC panel at the same field site, Royal Brighton Marina. Each day (run) for 8 days, 10 colonies were transported to the laboratory a day before measuring their feeding

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activity in the presence or absence of conspecific chemical cues (N=80). Each colony was collected by cutting the acetate sheet into 1×1 cm squares, and attached to the base of a 500 ml clear plastic container, filled with filtered seawater and aerated overnight at room temperature.

On the day of testing, five colonies were randomly assigned to the conspecific chemical cue treatment and five to a control treatment (untreated filtered seawater). The chemical cue was delivered using seawater pre-exposed to *B. neritina* colonies as described above. After 1.5 h of acclimatisation (a time based on maximum feeding behaviour measured in pilot studies), we recorded the maximum number of visible feeding structures, i.e. the extruded lophophores, observed under a microscope once every 25 s for a total of 9 times, although 29 colonies were measured only 8 times and three colonies between 5 and 7 times owing to missed recordings. All colonies were then exposed to untreated filtered seawater for 1.5 h before being assigned to the alternative treatment and their feeding activity was quantified again as above. For each colony, we calculated the difference in lophophore count between the chemical cue and control treatment. The size of each colony was recorded as the number of individual zooids visible.

Statistical analysis

All statistical analyses were performed in R (https://www.r-project.org/). In experiments 1 and 2, we used linear models to test for an effect of food (fed or starved), water treatment (oxygen depletion and disrupted flow for experiment 1, and chemical cues for experiment 2), run, colony mass (as a covariate) and any interactions among these factors on metabolic rate. In experiment 3, we used linear models to test for an effect of chemical cues, run and mass on metabolic rate. Because metabolism and mass scale allometrically, these two variables were $\log_{10}$-transformed prior to analyses. For experiment 4, we used linear mixed effects models to test whether the difference in lophophore count between control colonies and colonies exposed to conspecific chemical cues was significantly different from zero, including run and zooid number as fixed factors, and the order of exposure to the treatment as a random factor. Models were progressively reduced by removing non-significant interactions (P>0.20). In experiments 1 and 3, five colonies were excluded from the analyses as their metabolic rates were ∼0, indicating that the animals had died (data are accessible at https://doi.org/10.26180/5eec6125bf4e5).

RESULTS AND DISCUSSION

Experiment 1 – food and abiotic factors: oxygen depletion and disrupted water flow

Food and colony mass interactively affected colony metabolism so that metabolism increased faster with mass in fed colonies than in starved colonies ($F_{1,24}$=5.43, P=0.03; Table 1, Fig. S1). There was no effect of reduced oxygen availability or disrupted water flow on metabolic rates ($F_{2,24}$=0.53, P=0.6).

Experiment 2 – food and conspecific chemical cues

Consistent with experiment 1, we found an interaction between food treatment and colony mass ($F_{1,15}$=8.53, P=0.01): across the range of mass considered, fed colonies always had a higher metabolic rate than starved colonies, but the reduction in metabolism in the absence of food was stronger for smaller colonies. There was no evidence of an interaction between food and conspecific cues ($F_{1,15}$=1.88, P=0.19), but there was an indication that colonies exposed to chemical cues had lower metabolism than control colonies ($F_{1,15}$=3.71, P=0.07).

Table 1. Linear mixed effects model assessing the effects of food treatment (fed or starved), water treatment (control, oxygen-depleted or disrupted water flow), body mass ($\log_{10}$-transformed) and run on individual metabolic rate ($\log_{10}$-transformed) of *Bugula neritina* colonies (N=39)

	d.f.	F	P
Food treatment	1	4.752	0.039
Water treatment	2	0.530	0.596
Run	3	16.175	<0.001
Mass	1	32.260	<0.001
Food treatment: mass	1	5.430	0.029
Water treatment: run	6	2.689	0.039
Residuals	24		

Experiment 3 – the role of conspecific chemical cues

When we further explored the effects of chemical cues in starved colonies, we found a significant reduction in metabolic rate in colonies exposed to conspecific chemical cues in comparison to control colonies ($F_{1,38}$=13.29, P<0.001; Fig. 1).

Experiment 4 – is metabolic suppression linked to reduced feeding behaviour?

Feeding activity was unaffected by chemical cues as the difference in lophophore count between colonies exposed to conspecific chemicals and those unexposed was not different from zero (t_{79}=−1.23, P=0.22; Fig. 2).

We show that metabolic suppression in response to conspecifics is caused by two independent drivers: food availability and conspecific chemical cues. Changes in metabolism in response to food availability are expected because rates of food intake and metabolism are correlated. Animals that experience low food availability might therefore have lower metabolism because they are not processing food (e.g. SDA effects; Secor, 2009), but might also actively downregulate their metabolism as a strategy to conserve energy (Auer et al., 2015; Ghedini et al., 2017). Regardless, conspecific reductions in food availability are one of the drivers of metabolic suppression.

We show that metabolic suppression also occurs in response to conspecific chemical cues independently of food. Many marine

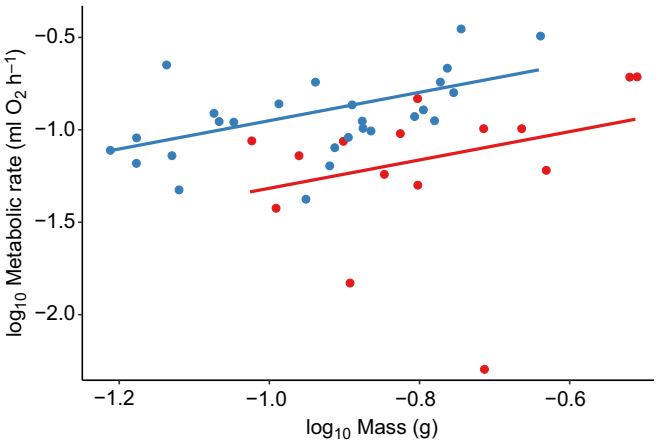

Fig. 1. Relationship between metabolic rate and mass for starved *Bugula neritina* colonies. Colonies exposed to conspecific chemical cues (red, N=15) have lower metabolic rates than control colonies (blue, N=27) across the entire range of mass considered (ANOVA, P<0.001). Each dot represents one colony and the lines represent predicted values from linear models. All data are $\log_{10}$-transformed.

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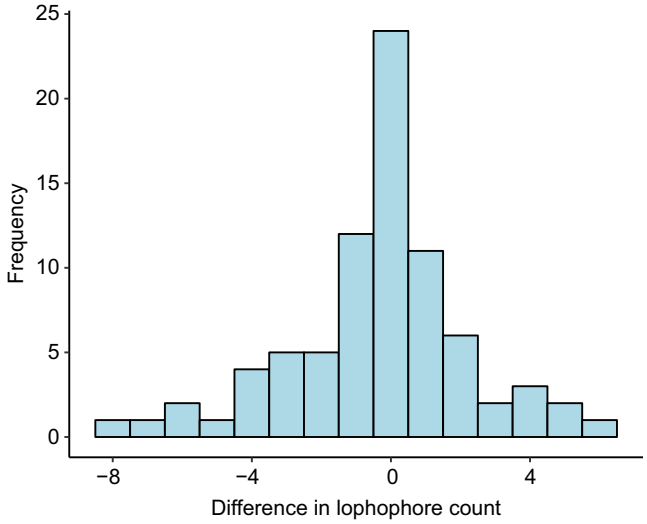

Fig. 2. Frequency distribution of the difference in average lophophore count between *Bugula neritina* colonies over eight runs (*N*=80) exposed to seawater with and without the presence of conspecific chemical cues. The difference in lophophore count between the two treatments was not different to zero (two-tailed *t*-test, $P>0.05$), indicating no effect of conspecific cues on feeding activity.

species rely on water-borne chemical cues such as primary metabolites and waste materials as a form of communication (Hay, 2009). These chemical cues from conspecifics (Nadler et al., 2016) or heterospecifics can reduce metabolism (Poulson-Ellestad et al., 2014). Here, we add to this evidence by showing that chemical cues drive metabolic suppression independently of food and that other conspecific effects on the environment, such as reductions in oxygen availability or water flow, do not affect metabolic rates, at least in our study species.

Conspecific chemical cues may signal environments of intense competition and hence low food availability, such that organisms respond by lowering their metabolic demands. It is surprising that these cues induce metabolic suppression independently of food availability. Phytoplanktonic food in the marine environment can, however, be highly unpredictable and change rapidly depending on currents or nutrient availability (Okamura, 1984). Therefore, particularly in sessile organisms, chemical cues might represent a more reliable source of information regarding the surrounding competitive environment than food availability itself at any moment in time. The sessile nature of these organisms might also explain why their metabolism is sensitive to conspecifics, while some mobile animals (e.g. *Daphnia*) do not consistently show density-dependent metabolism (Yashchenko et al., 2016). Nonetheless, metabolic suppression occurs in response to conspecifics both in mobile and sessile species (Poulson-Ellestad et al., 2014; Nadler et al., 2016; Ghedini et al., 2017; Malerba et al., 2017). To what extent differences among species (e.g. identity of chemical cues) and methodological differences (e.g. delivery or abundance of conspecific cues) mediate metabolic responses remains to be tested.

Although organisms can actively downregulate their metabolism in response to conspecific cues, these cues could also induce downregulation of metabolism in nearby individuals. Such allelopathic effects occur within (Sudatti et al., 2020) and among species (Inderjit et al., 2011; Poulson-Ellestad et al., 2014), although most documented effects are on growth and not metabolic rates directly (but see Poulson-Ellestad et al., 2014). The release of chemical cues that induce metabolic suppression can give organisms a competitive advantage by reducing the demand and acquisition of resources by neighbours (Bieberichid et al., 2018), but can also lead to autotoxic effects (Sudatti et al., 2020). Changes in metabolism might also occur where conspecific cues signal increased predation risk (e.g. from injured individuals); although we cannot rule out this possibility, we think it is a less likely explanation as stress signals tend to increase metabolism rather than reducing it (Gibson and Mathis, 2006; Janča and Gvoždík, 2017; Pereira et al., 2017).

Whether it is an active or induced response, metabolic suppression means that less energy is available for biological work (Killen et al., 2013). Contrary to our expectation, metabolic suppression was not linked to reduced foraging activity. Thus, metabolic suppression in the presence of conspecific cues reduced the amount of energy expended on physiological processes rather than foraging. Whilst searching for food might be a costly activity, the energy devoted to feeding effort might remain unchanged under competition as searching for food might become even more important to access resources when competition is intense.

Interestingly, changes in local abiotic conditions, either in the form of oxygen availability or water flow, did not trigger metabolic suppression. Although oxygen availability influences the small-scale distribution and abundance of organisms (Ferguson et al., 2013) and can reduce metabolic rate in other species (Nässberger and Monti, 1984), reductions in oxygen availability did not drive metabolic suppression in *B. neritina* in the present study. Importantly, we tested metabolic responses at oxygen levels above the critical P_{O_2} of the species (Lagos et al., 2017) to avoid oxygen limitation. However, reductions in oxygen often co-occur with changes in water flow, such that these two factors might act in synergy or exacerbate the effects of conspecific chemical cues. Therefore, metabolic suppression might be stronger where multiple environmental changes induced by conspecifics co-occur.

Density-dependent reductions in metabolic rate are associated with declines in maintenance, reproduction and population growth (Harvell et al., 1990; Delong and Hanson, 2009). Nonetheless, metabolic plasticity has benefits as it allows organisms to cope with short-term changes in environmental conditions (Norin and Metcalfe, 2019). Indeed, a reduced metabolism means both a lower energy requirement, which is more likely met via food intake, and a lower energy expenditure such that energy reserves are not depleted (Auer et al., 2015). However, the advantages of metabolic reductions are counter-balanced by the energy needs for survival and fitness, such that metabolic suppression is a trade-off between these two conflicting processes and might be advantageous only in the short-term (Burton et al., 2011; Ghedini et al., 2017). Therefore, whether metabolic suppression is an adaptive response to high population densities is unknown.

In conclusion, although conspecific cues can have positive or no effects on metabolism (Yashchenko et al., 2016; Pereira et al., 2017), individual organisms often display reduced metabolic rates in the presence of conspecifics (Delong et al., 2014; Nadler et al., 2016; Ghedini et al., 2017; Malerba et al., 2017). Here, we showed that food availability and conspecific chemical cues are two independent drivers of density-dependent metabolism. Our results highlight the role of conspecific cues as an important but overlooked source of variation in metabolism. The consequences of metabolic suppression for competitive outcomes remain largely unexplored, but are important to consider when estimating energy fluxes (Ghedini et al., 2020). Determining how widespread intraspecific and interspecific metabolic suppression are among species is an important missing piece of information to explain metabolic

variation and its consequences for competitive dynamics in populations and communities.

Acknowledgements
The authors thank the Royal Brighton Yacht Club for access to the field site. We also thank B. Comerford and M. Parascandalo for assistance with laboratory procedures.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: D.J.M., G.G.; Methodology: M.K.L., D.J.M., G.G.; Software: M.K.L.; Validation: M.K.L., D.J.M., G.G.; Formal analysis: M.K.L., G.G.; Investigation: M.K.L.; Resources: D.J.M., G.G.; Data curation: M.K.L.; Writing - original draft: M.K.L.; Writing - review & editing: M.K.L., D.J.M., G.G.; Visualization: M.K.L., D.M.; Supervision: D.J.M., G.G.; Project administration: M.K.L., D.J.M., G.G.; Funding acquisition: D.J.M., G.G.

Funding
This research was supported by the Australian Research Council (DE190100660) to D.J.M. and G.G.

Data availability
Data are accessible at https://doi.org/10.26180/5eec6125bf4e5

Supplementary information
Supplementary information available online at
https://jeb.biologists.org/lookup/doi/10.1242/jeb.224824.supplemental

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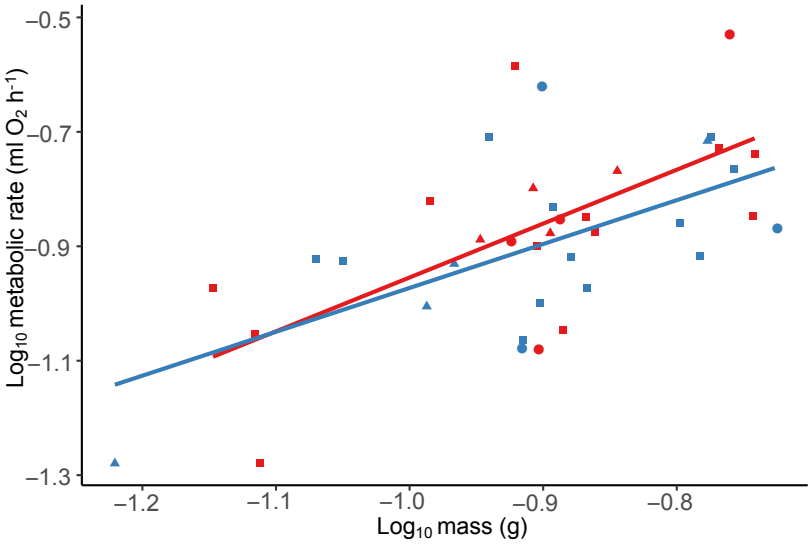

Fig. S1. Predicted relationship between metabolic rate and mass for fed (red line, N = 20) and starved (blue line, N = 19) colonies of *Bugula neritina*. Feeding had a significant effect on metabolic rate in *Bugula* colonies, (ANOVA, $p < 0.05$) independently of the other density-mediated treatments to which they were exposed (oxygen-depleted water = triangle, disrupted flow = circle, control = square), but the effects of food were mediated by colony mass: metabolic rate increased faster with mass in fed colonies (see also Table 1). All data are $\log_{10}$-transformed.