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Running head: Competition affects energy budgets

Does energy flux predict density-dependence? An empirical field test

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Abstract

Changes in population density alter the availability, acquisition and expenditure of resources by

individuals, and consequently their contribution to the flux of energy in a system. Whilst both

negative and positive density-dependence have been well studied in natural populations, we are

yet to estimate the underlying energy flows that generate these patterns and the ambivalent

effects of density make prediction difficult. Ultimately, density-dependence should emerge from the effects of conspecifics on rates of energy intake (feeding) and expenditure (metabolism) at the organismal level, thus determining the discretionary energy available for growth. Using a model system of colonial marine invertebrates, we measured feeding and metabolic rates across a range of population densities to calculate how discretionary energy per colony changes with density and test whether this energy predicts observed patterns in organismal size across densities. We found that both feeding and metabolic rates decline with density but that feeding declines faster, and that this discrepancy is the source of density-dependent reductions in individual growth. Importantly, we could predict the size of our focal organisms after 8 weeks in the field based on our estimates of energy intake and expenditure. The effects of density on both energy intake and expenditure overwhelmed the effects of body size; even though higher density populations had smaller colonies (with higher mass-specific biological rates), density effects meant that these smaller colonies had lower mass-specific rates overall. Thus, to predict the contribution of organisms to the flux of energy in populations it seems necessary not only to quantify how rates of energy intake and expenditure scale with body size, but also how they scale with density given that this ecological constraint can be a stronger driver of energy use than the physiological constraint of body size.

Keywords: competition, population, metabolic theory, trophic interactions, geometric biology, growth rate, respiration, homeostasis

Introduction

The need for generalization in ecology has prompted the development of unifying theories that offer a mechanistic understanding of how processes affect patterns allowing predictions across hierarchical levels (Levin 1992, Loreau 2010). Energy flows provide a particularly helpful pathway to link small scale processes to large scale patterns (Gillooly et al. 2001) and have a long tradition in ecosystem ecology (Lindeman 1942, Odum 1956, Pimm and Lawton 1977). Studies of energy flows have also been used to generalize individual-level processes of resource acquisition (Macarthur and Pianka 1966) and expenditure across species (Brown et al. 2004, Sousa et al. 2008). The rates at which organisms consume energy and materials have attracted considerable interest for the opportunity to scale these processes to population dynamics (Belgrano et al. 2002, Savage et al. 2004) and community structure (Petchey et al. 2008, Yvon-Durocher and Allen 2012). Nonetheless, whilst there are physical and biochemical constraints on the way organisms intake and expend energy (West et al. 2002), energetic processes cannot be considered independently of the ecological interactions in which they are nested. Where theory fails to account for the effects of such higher-level constraints on individual processes, predictions might not reflect patterns observed in nature (e.g. Chalcraft & Resetarits 2004).

Competition for resources imposes a key constraint on the availability, acquisition and use of energy by individuals and, consequently, on their growth and fitness. Under some conditions the presence of conspecifics can enhance survival and reproduction, favouring the persistence of a population (Allee 1931, Bertness 1989, Fajardo and McIntire 2011). Similarly, positive interactions among individuals of different species promote coexistence in resource-limited communities (Stone and Roberts 1991, Bertness and Callaway 1994, Gross 2008). At high densities, however, individuals often suffer reduced growth rates and fitness. Reduction in fitness

is typically attributed to competitive processes diminishing the availability of resources *per capita* (exploitative competition) or reducing access to resources (interference competition) (Antonovics and Levin 1980, Violle et al. 2010); such reduction in resource intake leaves less energy available for fitness-enhancing processes such as growth and reproduction (Sinclair et al. 1985, Jenkins et al. 1999). However, it is becoming increasingly apparent that the effects of conspecific density on an individual's scope for production are more complicated than was once thought.

Scope for production can be defined as the 'discretionary energy' that is available to an organism for growth, reproduction or other fitness enhancing functions (Kearney and Porter 2009, Buckley et al. 2014). Discretionary energy can thus be estimated by the difference between rates of energy assimilated (from food consumption in animals) and rates of energy expended on respiration (metabolic rate estimated by oxygen consumption). Across population densities, therefore, an individual's growth rate is determined by how rates of resource intake (feeding) change with population density relative to rates of energy expenditure (metabolism). However, most studies of bio-energetics focus explicitly on either one or the other process (i.e. feeding or metabolism) and rarely on both. In foraging ecology, population density is considered an important factor influencing the foraging strategy and behaviour of individuals (Davidson and Morris 2001, Svanbäck and Bolnick 2007). Whilst feeding rates for a specific resource are predicted to decline with density in competitive populations, often no specific prediction is made for how *per capita* metabolic rates change across densities and are implicitly kept constant, i.e. energy demands are assumed to remain unaffected by density (e.g. Enquist et al. 2003, Lopez-Urrutia et al. 2006) (Fig. 1 Hypothesis 1). Yet, at an individual level, feeding and metabolism often show a strong co-dependence since metabolism is fuelled by food ingestion and, *vice versa*,

feeding is enabled by metabolic activity (Hilleradams and Childress 1983, Schmoker and Hernandez-Leon 2003). Not only the two processes are co-dependent, but they are both affected by conspecific density. Research on metabolic theory has recently revealed density-dependent effects on metabolism where the presence of conspecifics reduces *per capita* metabolic rates as a consequence of competitive effects (e.g. reduced food availability; DeLong and Hanson 2009, DeLong et al. 2014, Malerba et al. *in press*) or behavioural effects (e.g. lower stress levels in aggregated animals; Waters et al. 2010, Nadler et al. 2016). Thus, the ways in which both energy intake (feeding) and energy expenditure (metabolism) are affected by density remains unclear. Yet even slight differences in the density-dependence of feeding and metabolism will yield very different outcomes for the organism overall.

Are feeding and metabolism equally density-dependent? If the presence of conspecifics equally affects both rates of food intake and metabolism, then these processes would decline with density with the same slope. The amount of discretionary energy would thus be constant across densities and individual performance would be independent of population density (Fig. 1 H2). In nature, however, we often observe positive or negative density-dependent dynamics of growth and reproductive success (Antonovics and Levin 1980, Sibly et al. 2005). Whilst several mechanisms might be responsible for these patterns (e.g. predator overwhelming, thermoregulation), from an energy perspective they suggest that feeding and metabolic rates do not show density-dependence with identical slopes. Two other scenarios are therefore possible. Feeding rates might decline faster than metabolic rates so that discretionary energy declines with density (negative density-dependence or competition; Fig. 1 H3). Conversely, *per capita* metabolic rates might decline faster than feeding rates, so that discretionary energy increases with density representing an instance of apparent facilitation (Fig. 1 H4). Because too few studies have

examined the density-dependence of metabolism and feeding simultaneously (DeLong et al. 2014), it remains difficult to predict which hypothesis applies.

The effects of population density on body size (Damuth 1981) further complicate predictions about density and energy use. Many biological rates scale allometrically with the size of an organism, including metabolic rates (West et al. 2002, Brown et al. 2004) and feeding rates (Vucic-Pestic et al. 2010, Rall et al. 2012). If both metabolism and feeding scale with body mass at an exponent less than one, then smaller individuals tend to have higher energetic costs per gram of mass relative to larger individuals. Hence, any density-mediated changes in body size will in turn affect mass-specific rates of both metabolism and feeding. For example, a 20% decrease in mass will yield a 3.5% increase in mass specific metabolic rate and a 7% increase in mass specific feeding rate in forest arthropods, for which metabolism scales at 0.84 and feeding at 0.68 of mass (Reichle 1968). Hence, how body size affects the discretionary energy available for production depends on the relative scaling exponents of feeding and metabolism with size. While general rules for such exponents exist, these exponents are often quite species- and context-specific (Bokma 2004, Glazier 2005, Killen et al. 2010) such that an informative comparison requires measurements to be performed for the same population under the same conditions.

Here we created experimental populations of varying density and deployed them into the field. We use a marine sessile invertebrate, the arborescent colonial bryozoan *Bugula neritina*, as a model system to test how *per capita* (i.e. per colony) and mass-specific rates of energy intake and expenditure vary with population density. Previous studies showed that intraspecific competition in this species is manifested as decreased colony feeding success (Okamura 1984) and reduced growth rates (Hart and Marshall 2013). Since population density affects feeding

rates and colony growth, we anticipate that metabolic rates will also show density-dependence. This model system is, thus, ideal to test whether rates of energy intake though feeding and energy expenditure through metabolism have different density-dependence, i.e. decrease with density with different slopes. Furthermore, by simultaneously quantifying energy intake and expenditure, we were able to estimate discretionary energy per colony and use these estimates as predictors for body sizes across densities.

Methods

Experimental methods

The sessile colonial bryozoan *Bugula neritina* was used as a study organism to investigate the mechanisms by which population density influences per colony feeding and metabolic rates. We experimentally manipulated population density of colonies of *Bugula* by settling increasing number of larvae on experimental plates. After metamorphosis these larvae developed into adult colonies that were grown in the field at the different densities ranging from 1 to 30 individual colonies per plate.

We obtained larvae from adult colonies collected in the field (Brighton, Victoria, Australia) following standard procedures for spawning (Marshall et al. 2003) and settled larvae on roughened PVC plates ($7.5 \times 7.5 \times 0.5$ cm) to establish populations of varying densities. We had eight target population densities, ranging from 1 to 30 colonies per plate. These densities overlap with densities typically observed in the field without exceeding them (field observations: 0-0.75 colonies cm^{-2}, Hart and Marshall 2013; densities in this experiment: 0.02-0.53 colonies cm^{-2}). Each of the eight densities was replicated three times for a total of 32 plates that were randomly

arranged on a PVC backing panel ($55 \times 55 \times 0.8$ cm). On the same panel, we also deployed eight control plates that were treated the same way, but no larvae were settled on them and were kept clean from organisms except biofilm. We fully replicated this design on four panels with plates that were independently settled with larvae and deployed in the field at an interval of a week. Panels were suspended from floating pontoons (Blairgowrie, Victoria, Australia) at a depth of 1.5 m in a facedown horizontal orientation. Target densities were created two weeks after deployment and were maintained by removing new recruits of *Bugula* and other organisms each week. Populations on each panel were allowed to develop in the field for two months prior to taking them back to the lab for testing. Panels were tested at an interval of a week following the same order of deployment.

On the day before testing, plates with colonies were collected from the field and transported back to the lab where they were kept in aerated seawater tanks for ~ 20 hours without food prior to tests. Unhealthy (discoloured or evidently preyed upon) colonies were eliminated and densities of healthy colonies recorded for each plate. These densities differed from the initial target densities because of natural mortality occurring in the field. Nonetheless, densities ranged from 1 to 30 colonies per plate and all panels represented a range of low to high densities, specifically from 1 to 16 colonies for panel 1 and 2; 1 to 26 for panel 3; 1 to 30 for panel 4. Plates were cleaned with a brush and spatula to eliminate organisms, other than our focal *Bugula* colonies. Control plates were used to account for the effect of biofilm that could not be completely scraped off plates. Plates were then randomly allocated to two sets of 9 plates (7 plates for panel 1) representing populations from low to high densities; each set was run with three control plates, except for panel 1 that was run with four controls. Testing for both feeding and metabolism occurred on the same day. After testing, we measured total population mass by blotting dry the

colonies on each plate and weighting them to the nearest 0.01 g and subtracting the known plate mass. Per colony rates of feeding and metabolism were calculated by dividing population-level feeding and metabolic rate, respectively, by the density of colonies on each plate. Similarly, average colony size was estimated from total biomass and population density.

Estimating energy intake

The bryozoan *Bugula neritina* is a suspension feeder that actively filter plankton from seawater (Allen et al. 2008). In laboratory testing, populations of *Bugula* were fed with cultures of the unicellular green alga *Dunaliella tertiolecta* that were grown using a standard enriched seawater medium (F/2 medium solution without silica; Guillard and Ryther 1962, Guillard 1975) at controlled temperature conditions (20˚C). Algal cultures were grown for a month prior to tests and were re-inoculated weekly to maintain a relatively similar phytoplankton concentration across weeks. Prior to each feeding run, the concentration of algae in the cultures was determined *via* optical density and these values were used to determine the appropriate algal biovolume to use each week to account for variation in concentration across weeks.

Feeding rates were measured in 12 acrylic water baths ($16 \times 13 \times 5$ cm), each connected to a small peristaltic pump (Kamoer KSP-F Series dosing pump, Kamoer fluid tech, Shanghai Co., Ltd.) that recirculated water for the duration of the feeding trial. Each bath was prefilled with 0.6 litres of filtered seawater and pumps were started to initiate the water flow. The volume of algae was then added to each bath (60 ml for panel 1 and 2; 75 ml panel 3; 85 ml for panel 4; these different volumes were based on measurements of optical density and accounted for variation in phytoplankton concentration). Samples to determine initial phytoplankton abundance (10 µl) were taken after 5 minutes to allow enough time for algae to mix with the seawater in the bath.

An experimental plate was then added to each bath and animals were left feeding for three hours, covered to minimize disturbance. At the end of the three hours water samples were collected again to estimate population consumption (initial – final phytoplankton concentration). Algae in samples were immediately fixed in a 2% Lugol solution and phytoplankton concentration was then estimated by manual cell counts (Neubauer haemocytometer). The second set of plates of panel 4 was excluded from the analyses because initial explorations revealed that colonies had consumed almost all the phytoplankton present in the baths before we sampled, so we risked making gross underestimates of consumption rate. Four additional data points were excluded from the analyses because estimates of per colony consumption rates were negative. The total number of colonies used for feeding analyses was N = 55.

Estimating energy expenditure

Population metabolic rates were estimated using a similar set-up used for feeding measurements, consisting of 12 acrylic hermetic water baths each connected to a peristaltic pump to ensure continuous water flow. Each bath was used as a respirometer chamber that was completely filled with sterilised filtered seawater and sealed with a lid after the plate with animals was put inside. Changes in % air saturation were determined using an optical oxygen meter (FireStingO2, Pyro Science GmbH, Germany) and flow-through cells with oxygen sensor (Pyro Science GmbH, Germany) placed in the tubing connecting the bath to the peristaltic pump. Animals were left to acclimate for 15-20 minutes prior to starting the measurements which run for three hours. The rate of oxygen consumption (V_{O2}, ml h^{-1}) was calculated following Alton et al. (2007) using the equation:

$$V_{O2} = -1 \times [(m_b - m_c) / 100] \times V \times \beta_{O2}$$

where m_b is the slope of the line relating oxygen saturation to time for the baths containing

Bugula populations (% air-saturation h^{-1}), m_c is the slope for the control baths (% air-saturation

h^{-1}), *V* is the water volume in the baths minus that taken up by the plate and the colonies (litres)

and βO_2 is the oxygen capacitance of air-saturated seawater at the specific temperature and

salinity of the run (ml l^{-1}; exact value varied according to the temperature of the run, see

Appendix 2 in Cameron 1986. Experimental runs were done in a temperature controlled room.

Temperature was allowed to increase slightly from the first to the last test of panels because field

temperatures were increasing during the course of the experiment (panel 1 = 15.7˚C; panel 2 =

16.7˚C; panel 3 = 18.7˚C; panel 4 = 18.4˚C ± 0.001 ˚C for all panels). Across the four panels the

total number of *Bugula* colonies used for analyses was N = 65.

Statistical analyses and energy calculations

We used generalized linear mixed models (GLMM) to test how average colony mass, population

density and their interaction influenced per colony feeding and metabolic rates. For the analyses

both predictors and responses were log_{10}-transformed, except for density. Average colony size

and density were considered as continuous fixed effects, whilst panel was included in the

analyses as a categorical random effect. Models were fitted using maximum likelihood and were

reduced by eliminating non-significant interactions ($P > 0.05$).

The equations and parameter estimates obtained from mixed model analyses were used to

estimate feeding and metabolic rates per colony, which were then converted into values of

energy intake (feeding) and expenditure (metabolism). Energy intake was calculated from

estimates of phytoplankton consumption by converting the known carbon content of

phytoplankton (2.85×10^{-8} mg of C $cell^{-1}$) to calories, where 1 mg of C approximates 11.4

calories (Platt and Irwin 1973). Since food ingested is only partially assimilated and converted into energy, we assumed a 50% assimilation efficiency (Targett and Targett 1990) to avoid overestimating energy gains. Whilst marine animals display a range of assimilation efficiency rates (i.e. 80% to 7%; Lobel and Ogden 1981, Rubilar et al. 2016), crucially using a different estimate would not change the slope at which feeding rate changes with density, the parameter of key interest. Energy expenditure was calculated by multiplying the estimates of population metabolic rates for the conventionally assumed thermal equivalent of O_2 exchange with proteins as main metabolic substrate (i.e. 19 kJ l^{-1}; Walsberg and Hoffman 2005). By subtracting energy expenditure (metabolism) from energy intake (feeding) we obtained estimates of discretionary energy per colony (i.e. delta, J h^{-1}; N = 59). We used linear mixed models (with panel as categorical random effect) to assess how discretionary energy and average colony size changed with population density and to test whether estimates of discretionary energy could predict the observed changes in colony size across densities. Finally, we compared the trajectory of change in mass specific metabolic rates across densities predicted from the scaling of metabolism with body mass alone (i.e. according to metabolic theory) with the observed change in mass specific metabolic rates predicted from the combined effects of mass and density on metabolism. All analyses were performed in SYSTAT (Systat Software, San Jose, CA).

Results

Both per colony assimilation (energy intake from feeding) and respiration rates (oxygen consumption) displayed negative density-dependence, but the decline in food intake was greater than the decline in metabolic rate with density (Fig. 2a and b, Table 1). Rates of energy intake

and expenditure per colony were also affected by the average size of colonies, with both rates increasing with body size (slope: 0.465 for feeding and 0.626 for metabolism; Table 1).

The amount of discretionary energy per colony (delta), calculated as the difference between estimates of energy intake and expenditure, declined with density (Fig. 2c, Table 2). Importantly, these estimates of discretionary energy were good predictors of variation in colony size across population densities, with size increasing with discretionary energy with a slope of 0.05 ($F_{1,54} = 113.39$, $P < 0.001$; Fig. 2d, Table 2).

Since average colony size declined with density (Appendix S1: Fig. S1, Table S1) and metabolism scales allometrically with mass in this species (Barneche et al. 2017), mass specific metabolic rates were anticipated to increase with density when considering body size alone (Fig. 3); that is because the smaller colonies found at higher densities would be predicted to have a higher mass specific oxygen consumption relative to larger colonies in sparser populations. Interestingly, when considering both the effects of body size and density on metabolism, mass specific metabolic rates declined with density despite the declining size of colonies (Fig. 3). The decrease in metabolic rate per unit mass was accompanied by a decline in rate of food intake per unit mass with density (Appendix S1: Fig. S2, Table S2). However, as observed for whole colony rates, the decline of feeding and metabolism was not equal; mass specific feeding rates declined faster than mass specific metabolic rates such that the amount of discretionary energy per gram of mass (i.e. mass specific discretionary energy) decreased with density (slope -0.024; $F_{1,54} = 114.71$, $P < 0.001$; Appendix S1: Fig. S2, Table S2).

Discussion

An individual gains energy *via* feeding (assimilation) and expends this energy on metabolic expenditure on respiration and production (growth and reproduction). Hence, it is reasonable to expect a strong co-dependence between rates of energy intake and rates of energy expenditure (DeLong et al. 2014). Here we show that, while both feeding and metabolism decline with density, their rate of decline differs (Fig. 1 H3). The higher sensitivity to density of feeding relative to metabolic rates accounts for density-dependent growth in our system and offers a mechanistic understanding of why individual growth and reproductive output decline with population density (Bohlin et al. 2002, Sibly et al. 2005). Most importantly, the difference between energy intake and energy expenditure at the individual colony level was a good predictor of the average body size in populations of varying densities.

In resource limited environments, the presence of conspecifics causes patterns of negative density-dependent growth by reducing the availability of food *per capita* (Damuth 1981, Amundsen et al. 2007). Our experimental populations showed similar patterns of density-dependence; both food consumption and average body size declined with density indicating that food was a limiting resource for which individual colonies were competing. This results is in accordance with results from field populations of *Bugula*, where the abundance of food affects colony growth and mediates density-dependent effects (Svensson and Marshall 2015), driving reductions in body size (Hart and Marshall 2013) and fitness with increasing conspecific densities (Allen et al. 2008).

While declines in food consumption with density were expected, declines in metabolic rate were more surprising because the effects of density-dependence on metabolic rates have not yet been incorporated into theory to allow clear predictions. Nevertheless, it should be adaptive for

organisms to respond to density, competition or ecological processes that similarly affect assimilation by modifying their respiration rates and reallocating energy between respiration (e.g. reducing maintenance costs) and production (e.g. reducing discretionary energy expenses for biomass production). So why does metabolic rate decline with density? Given the co-dependence of feeding and metabolism (Schmoker and Hernandez-Leon 2003), a reduction in food intake with increasing conspecific density is likely accompanied by a reduction in metabolic rate at the individual level (DeLong and Hanson 2009). In our experiment, feeding and metabolism were deliberately measured in separate trials which were randomized in order (i.e. some colonies were fed before metabolic measurements, others after) and seawater used for metabolic measurements was free of food sources (i.e. filtered and sterilized). The observed reductions in metabolic rate could, thus, not be caused by instantaneous competitive reductions in food intake, since food was not supplied in measurements of metabolic activity. Nonetheless, it is possible that food competition experienced in the field has a longer lag effect on colonies, such that metabolic rate is reduced in the long term.

Metabolism in animals is not only a reflection of food intake, but can also be actively adjusted to environmental conditions (Guppy and Withers 1999, Sousa et al. 2010). For example, metabolic depression has been recorded in most animal phyla as a response to environmental stress or food deprivation (Sokolova et al. 2012, Jager et al. 2016). Conversely, organisms that rely on blooming resources can accelerate the rate at which energy is consumed to maximize consumption of these resources, whilst remaining small and responding quickly in population numbers (Kooijman 2013). The presence of conspecifics can, similarly, elicit changes in an individual's metabolic activity (Waters et al. 2010, Nadler et al. 2016) resulting in reduced metabolic rates (DeLong et al. 2014, Malerba et al. *in press*) as well as reduced energy expended

on growth, reproduction and associated discretionary expenses (e.g. courtship, mating, territorial defense, exploratory behaviors; Grant & Porter 1992). Our model species (i.e. the bryozoan *Bugula neritina*) is sessile and does not display obvious behaviours, but colonies can detect the presence of conspecifics in their surrounding (Gooley et al. 2010) and their density can alter the morphology of colonies and their feeding structures (Thompson et al. 2015). This ability to perceive and react to conspecifics suggests that, similarly to other species, colonies might actively reduce metabolic rates and thus production rates as population density increases. While the cue that induces this plasticity is unknown, changes in local oxygen levels are a good candidate (Ferguson et al. 2013). Importantly, our results suggest that the decline in *per capita* (i.e. whole colony) metabolic rate was an active physiological response rather than being a result of oxygen limitation. Metabolic suppression in *Bugula* occurs when oxygen levels reach 25-30% of oxygen saturation, whilst the percentage of oxygen saturation during our measurements varied between 100% and 60%, thus remaining well above levels that cause metabolic suppression in this species (Lagos et al. 2017).

Importantly, our estimates of discretionary energy per colony accurately predicted variation in body size among densities. This result highlights that both processes (i.e. feeding and metabolism) need to be measured to predict scope for production since their differential change with density determines the discretionary energy available for growth (Fig. 1). The steeper decline in feeding relative to metabolism provides a mechanistic understanding of patterns of negative density-dependent growth often observed in nature and offers a framework to understand how this pattern can shift to positive density-dependence (Bertness 1989, Fajardo and McIntire 2011). Facilitation can occur where the presence of conspecifics causes slopes of feeding and metabolism to diverge, thus increasing discretionary energy available for growth

with density (Fig. 1 H4). Local resource availability may mediate the relative importance of competitive and facilitative processes in natural populations (Svanfeldt et al. 2017). It may be that conspecific facilitation occurs when metabolism declines with density but locally abundant resources means that energy intake does not decline – this prediction awaits testing.

Density-mediated changes in size not only influenced rates of resource use at the whole colony level but also resource use per gram of mass (i.e. mass specific rates). Since metabolism typically scales allometrically with mass (Brown et al. 2004), we predicted that mass specific energy usage should increase as an organism gets smaller in denser populations. Interestingly we observed the opposite pattern, i.e. a reduction in mass specific metabolic rate for the smaller colonies in crowded populations (Fig. 3). This result suggests that colonies reduced their energy use per gram of mass in the presence of conspecifics beyond predictions based on their body size alone. Thus, the effects of conspecific density overwhelmed the effects of body size in determining mass specific rates of energy use. Such reduction in energy use could result in slower production as metabolism provides power for biological work, but it could be advantageous in dense populations when food intake is reduced due to resource depletion (Kooijman 2013). Nonetheless, metabolic reductions are limited to some extent (i.e. metabolism needs to occur at a minimum level that satisfies maintenance needs) and might not be beneficial in the long term. Whilst reduced metabolism can be beneficial to cope with environmental stress or survive at high conspecific densities, having a persistently low metabolic rate might restrict the capability of an individual to invest energy in processes that enhance fitness (e.g. cope with disease, defence mechanisms or synthesis of reproductive organs). This perhaps explains why individuals only reduce their metabolic rates when facing reduced resource availability; when resources are abundant, it might still be beneficial to have a higher basal metabolic rate.

Metabolism is considered a 'unifying process' in ecology because it connects all levels of ecological organisation by setting rates of demand and use of resources (Savage et al. 2004). Here, we experimentally demonstrate that density-dependent growth of individuals can be explained by the steeper decline of energy intake (feeding) relative to expenditure (metabolism). Furthermore, we show that conspecific density can influence the discretionary energy available for production not only by altering availability of resources per colony, but also by causing changes in mass specific rates of energy use that could not be anticipated by the scaling of metabolism with body mass alone. The rates at which organisms consume energy and materials provide a common currency to link processes across scales (Enquist et al. 2003, Ghedini and Connell 2016). Nevertheless, the physiological constraints of body size on energy use might be overwhelmed by ecological constraints (e.g. population density). Accounting for the mediating effects of such higher-level interactions on energy budgets might thus be necessary for predicting how individual energy use contributes to the flow of energy in populations and communities.

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Table 1. Linear mixed model estimating the effect of average colony mass and density on (a) per

colony feeding (N = 55) and (b) per colony metabolic rate (N = 65). All data are $\log_{10}$-

transformed except for density. Significant effects are reported in bold.

Source	Parameter	95% CI		df	t	F-ratio	p-value
		Lower	Upper				
(a) Feeding							
Fixed effects							
Intercept	1.231	0.828	1.634	3	6.143		**0.009**
Average mass	0.465	0.101	0.829	49	2.568	6.595	**0.013**
Density	-0.027	-0.039	-0.015	49	-4.437	19.691	**0.000**
Random effects							
Panel	0.019						
Residual	0.079						
(b) Metabolism							
Fixed effects							
Intercept	-1.002	-1.223	-0.781	3	-9.062		**0.003**
Average mass	0.626	0.424	0.827	59	6.22	38.640	**0.000**
Density	-0.013	-0.021	-0.006	59	-3.499	12.240	**0.001**
Random effects							
Panel	0.002						
Residual	0.046						

Table 2. Linear mixed model estimating the effect of (a) population density on estimates of discretionary energy per colony (i.e. delta, data are $\log_{10}$- transformed, N = 59) and (b) testing if estimates of discretionary energy predict average mass (N = 59). Significant effects are reported in bold.

Source	Parameter	95% CI Lower	Upper	df	t	F-ratio	p-value
(a) Delta							
Fixed effects							
Intercept	0.353	0.251	0.456	3	6.89		**0.006**
Density	-0.037	-0.04	-0.033	54	-21.96	482.05	**0.000**
Random effects							
Panel	0.009						
Residual	0.007						
(b) Average Mass							
Fixed effects							
Intercept	0.036	0.001	0.071	3	2.07		0.130
Delta	0.05	0.041	0.06	54	10.65	113.39	**0.000**
Random effects							
Panel	0.001						
Residual	0.001						

Figure 1. Predictions of how energy intake (feeding; green line) and expenditure (metabolism; orange line) might change with population density and the consequences for discretionary energy (grey shaded area). If feeding rates decline with density, but this has no effect on metabolism (H1), discretionary energy declines rapidly with density. If both feeding and metabolism decline with the same slope, discretionary energy remains constant across densities (H2). Density-dependence, however, suggests that feeding and metabolism might decline with density with different slopes. If feeding declines faster than metabolism, then discretionary energy declines with density (H3). Conversely, if metabolism declines faster than feeding, discretionary energy might increase with density (H4).

Figure 2. Predicted lines of best fit per panel from mixed effect models (with 95% CI) for the relationship between $\log_{10}$ per colony (a) feeding (N = 55) and (b) metabolic rate with density (N = 65). Both feeding and metabolic rates showed negative density-dependence, but the decrease in feeding rates (slope $-$ 0.027) was steeper than the decrease in metabolic rates ($-$ 0.013). (c) Since energy intake decreases faster than energy expenditure, discretionary energy declines with density (N = 59). (d) These estimates of discretionary energy predict average colony size observed across population densities, with colonies reaching larger sizes as discretionary energy increases (N = 59). Data points represent observed values and each line represents a panel with a common slope and a unique intercept.

Figure 3. The allometric scaling of metabolic rate with mass (exponent < 1) predicts mass specific metabolic rate to increase with density (predicted, black line) because of the density-mediated reduction in average colony size (Appendix S1: Fig. S1, Table S1); i.e. smaller colonies have higher mass specific metabolic rates than larger colonies. However, when both the

effects of density ad body size on metabolic rate are taken into account, mass specific rates

decline with density despite the smaller size of colonies (observed, orange line).

H1: discretionary energy declines but metabolism is constant

H2: constant discretionary energy

H3: discretionary energy declines

H4: discretionary energy increases