MONASH University

The Causes and Consequences of Variation in Metabolic Rate in a Sessile Marine Invertebrate

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A thesis submitted for the degree of Doctor of Philosophy at

Monash University in 2020

School of Biological Sciences

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"*[...] one general law, leading to the advancement of all organic beings, namely, multiply, vary, let the strongest live and the weakest die.*"

Charles Darwin, 1859

Abstract

Metabolic rate varies at all levels – among species, populations, and conspecifics, even after accounting for differences in body size, temperature, age, and sex. Over the last few decades, metabolic theory has proposed several hypotheses to explain this variation in metabolic rate in natural populations. Although we are starting to understand the drivers and the maintenance of variation in metabolic rate over time, we know little about its consequences for individual- and population-level processes, particularly under field conditions. In this thesis, I provide new insights into the drivers of variation in metabolic rate and its consequences for both individual performance and population-level dynamics in a marine colonial invertebrate, *Bugula neritina*. First, I investigate the effects of varying food availabilities and different environmental conditions on metabolic rate. Although metabolic rate is plastic, I show that such phenotypic plasticity is not necessarily adaptive by providing estimates of metabolic plasticity combined with formal estimates of phenotypic selection in two distinct environments in the field. Second, I test for the effects of metabolic rate on individual performance as well as population demography and intraspecific competition in the field. Here, I show that metabolic rate can interact with other traits such as body size and traits that determine resource acquisition to affect both individual performance and population-level processes. Overall, my work presents novel insights into how metabolic rate can affect individual-level processes and how these processes can scale up to affect population-level dynamics in the field.

Publications during enrolment

Schuster L, White CR, Marshall DJ (2019) Influence of food, body size, and fragmentation on metabolic rate in a sessile marine invertebrate. *Invertebrate Biology*, 138 (1): 55-66

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original paper published in a peer reviewed journal (Chapter 2) and one submitted publication (Chapter 3). The core theme of the thesis is the causes and consequences of variation in metabolic rate. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Biological Sciences under the supervision of Dustin J. Marshall.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapters 2, 3, 4, and 5 my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status** *(published, in press, accepted or returned for revision, submitted)*	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author(s), Monash student Y/N***
2	Influence of food, body size, and fragmentation on metabolic rate in a sessile marine invertebrate	Published	75%. Concept, design, data collection, data analysis, writing of manuscript	20% D.J. Marshall. Concept, design, analysis, edits to manuscript 5% C.R. White. Concept, edits to manuscript	No No

3	Plastic but not adaptive: environmentally-driven differences in metabolic rate despite consistent selection	Submitted	75%. Concept, design, data collection, data analysis, writing of manuscript	20% D.J. Marshall. Concept, design, analysis, edits to manuscript 5% C.R. White. Concept, design, edits to manuscript	No No
4	Testing predictions of metabolic theory: does metabolic rate affect population demography in the field?	Not submitted	73%. Concept, design, data collection, data analysis, writing of manuscript	20% D.J. Marshall. Concept, design, analysis, edits to manuscript 5% C.R. White. Concept, design, edits to manuscript 2% H. Cameron. Design, edits to manuscript	No No Yes
5	Metabolic phenotype mediates the outcome of competitive interactions in a response-surface field experiment	Not submitted	80%. Concept, design, data collection, data analysis, writing of manuscript	15% D.J. Marshall. Concept, design, analysis, edits to manuscript 5% C.R. White. Concept, edits to manuscript	No No

I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student name: Lukas Schuster

Student signature: **Date:** 20/10/2020

I hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the

responsible author, I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor name: Dustin Marshall

Main Supervisor signature: **Date:** 20/10/2020

Acknowledgements

This research was supported by the Monash International Postgraduate Research Scholarship (MIPRS). I was also fortunate enough to obtain an additional scholarship from Monash University that allowed me to cover my living expenses during my enrolment. However, this thesis would not have been possible without the support of many friends, family members, and colleagues, some of whom I have encountered along the way and who have always supported me. No words can express my appreciation for their love and words of support.

First and foremost, I would like to express my gratitude to my supervisor, Dustin Marshall, who gave me the opportunity to pursue a Ph.D. in Evolutionary Ecology and Physiology in spite of my background in Marine Biology. Over the years, I very much appreciated your time, patience, and words of encouragement, as well as the numerous challenges and opportunities which helped me grow as a scientist. When I first started, I had very little knowledge about Evolutionary Theory and Physiology, and three years and nine months later I am very proud to have completed a Ph.D. thesis in this field. I am incredibly grateful for your invitation to pursue this formative part of my academic journey in your lab – I have learnt so much from you!

I am also very grateful to my co-supervisor, Craig White. Thank you for always helping me out in difficult times and for our lovely science and non-science related chats in and outside of university. Having great interlocutors, such as you and Dustin, made me grow both as a scientist and a person.

I would also like to thank my panel members: Keyne Monro, Matt Hall, and Damian Dowling. You all provided lots of support and encouragement throughout the years. My

special appreciation goes to Keyne who was very encouraging from the very beginning. Your kind words always made me feel confident that I was on the right path and that one day I would be able to achieve my goals. I also very much enjoyed our nights out in Balaclava; hopefully there will be many more to come!

My utmost gratitude goes to Hayley who has been my guardian angel for years. What would I have done without you? Whenever I needed help, you were there for me. All the hours we spent together trying to make sense of analyses and having scientific discussions. A big shout-out also goes to all the other MEEGers – Martino, Giulia, Mariana, Belinda, Michaela, Amanda, Karin, Evatt, Annie, Liz, Lucy, Tormey, Emily, Alex, Mel, George, Sam – and the non-MEEGers who were somehow also part of our big MEEG family – Piet, Monica, Jules, Carmen, and Candice – without your support I could have never made it this far! Thank you for all the fruitful discussions at work and the fun times during after-work hours. Special thanks go to Belinda who carried the lab on her shoulders for so many years. Without you, nothing would have been possible. I will never forget our amazing field trips, especially our first *Bugula* collection! How much we laughed – such sweet memories!

It goes without saying that I have also received a lot of academic and administrative support from various other people in the School of Biological Sciences: Fiona Hibbert, Leesa Hughes, Barbara Hardie, Josie Powell, and Dani Annese!

Last but not least, a big thank you goes to all my friends outside of Australia who have been sending their support from overseas. Heartfelt thanks to Lucia who is always there in good and bad times, pushing me to do better and to achieve my goals! Thanks for all the LOLs

over the years (so viel Spaß sollte ja bekanntlich verboten werden) and the huge quantities of Mannerschnitten, which were an important source of fuel during my Ph.D.!

A riesigis Danke an meina Elton, de wosn des olls erst meglich gimocht hom. A groaßis Danke vor ollem an die Mama – uhne deina Untostützung wari nia sovl weit kem!

Chapter 1

General introduction

Metabolic rate determines the rate at which organisms transform resources from the environment into energy and use this energy to live (Hulbert and Else 2000, Brown et al. 2004, Auer et al. 2018, Pettersen et al. 2018, but see Glazier 2015). The rate at which organisms use energy, however, varies at all levels – among species and populations, and even among conspecifics (Burton et al. 2011, Konarzewski and Książek 2013, White and Kearney 2013). Among species, variation in metabolic rate can be mostly explained by differences in body size – with body mass generally explaining >90% of the observed variation (White and Kearney 2013, White et al. 2019). The strong relationship between metabolic rate and body mass, however, conceals a great deal of variation in metabolic rate, and species of the same size can vary up to several-fold in their metabolic rates (White and Seymour 2004, White et al. 2019). Also within a species, metabolic rates have been shown to vary up to three-fold among conspecifics, even after accounting for differences in body size, temperature, age, and sex (Burton et al. 2011, Konarzewski and Książek 2013). Given that individuals have a finite energy budget, which they must allocate among growth, reproduction, and self-maintenance (Stearns 1992), trade-offs among these functions mean that variation in energy usage will likely have implications for life-history traits and hence fitness. Over the last few decades, metabolic theory has sought to explain the drivers of this variation in metabolic rate within natural populations (Glazier 2005, Burton et al. 2011, Pettersen et al. 2018).

Although we are starting to understand the causes of variation in metabolic rate among individuals and its maintenance within a population over time (Pettersen et al. 2018), little is known about the consequences of this variation in metabolic rate for ecological and

evolutionary processes – particularly for individual performance (such as survival, growth, and reproduction) and population demographic processes. Therefore, the overarching aim of this thesis was to investigate the consequences of intraspecific variation in metabolic rate for processes at both the individual (Chapters 2 and 3) and the population level (Chapters 4 and 5). To do so, I took advantage of the sessile nature of the marine colonial bryozoan *Bugula neritina*, which is commonly found as part of fouling communities throughout the world. Once *B. neritina* colonies reach maturity, they form clearly visible brooding structures called ovicells, and each ovicell broods a single larva (Woollacott and Zimmer 1975). Due to their sessile nature and their clearly visible ovicells, I was able to follow the performance (i.e. survival, growth, and reproductive outputs) of single individuals and populations in the field. An important first step, however, was to determine how metabolic rates vary with three key factors: body size, food availability, and fragmentation, in this species (Chapter 2).

Patterns and processes of variation in metabolic rate

A great deal of the variation in metabolic rate both among and within populations can be attributed to differences in environmental conditions. Metabolic rate, like many traits, is plastic; it changes when conditions change (reviewed in Norin and Metcalfe 2019). One of the most obvious and perhaps strongest drivers of such metabolic plasticity is temperature but there are many others (Clarke 2017). For example, metabolic rate is strongly dependent on resource availability, with individuals typically increasing their metabolic rates if resources are abundant and decreasing them if resources are scarce (Guppy and Withers 1999, O'Connor et al. 2000, Mueller and Diamond 2001, Naya et al. 2009, Schimpf et al. 2012, Auer et al. 2015). Given that individuals obtain energy from feeding, resource availability can be considered a key factor affecting the life history of an individual (Stearns 1992).

In order to quantify the effect of feeding on metabolic rate, physiologists often determine an individual's specific dynamic action (or SDA; Rubner 1902) – the postprandial increase in metabolic rate and the duration of an elevated metabolic response post feeding (reviewed in Secor 2009). To date, only few studies have investigated SDA in marine invertebrates, and only one study has quantified the effects of feeding on metabolic rate in a modular organism (Szmant-Froelich and Pilson 1984). Quantifying SDA in modular species may be of particular interest because of the way colonial individuals take up and transform resources in comparison to unitary individuals, given that colonial organisms consist of functionally autonomous or semi-autonomous, multicellular modules (Vuorisalo and Tuomi 1986). Here, the rate at which individuals take up resources often scales isometrically with body size (Okamura 1984, 1985, Pratt 2005), whereas metabolic rate scales allometrically with body size in most modular species (reviewed in Burgess et al. 2017). Consequently, the rate at which different sized individuals take up resources differs from the rate at which they transform resources and use energy.

In metabolic theory, one of the most studied relationships is the scaling of metabolic rate with body size, particularly since metabolic rate scales with body mass at an exponent less than 1 in most species (i.e. hypo-allometric scaling; Kleiber 1932, Savage et al. 2004, Burgess et al. 2017, White et al. 2019). Importantly, by estimating the relationship between metabolic rate and body size, differences in metabolic efficiencies associated with body size can be estimated, thereby also allowing for predictions of metabolic rates across all levels of organization (Brown et al. 2004). Most studies investigating metabolic scaling, however, necessarily rely on the natural variation in body size among individuals. Body size, in turn, is correlated with a range of other traits such as age and nutrition, which can confound estimates of metabolic scaling (Calder 1984, Schmidt-Nielsen 1984). Here, modular organisms such as *B. neritina* provide an opportunity to manipulate size independently of

other factors – thereby also allowing for direct tests of predictions of competing metabolic theories (Nakaya et al. 2005, White et al. 2011, Barneche et al. 2017, Burgess et al. 2017). Therefore, the main aims of Chapter 2 were to (i) quantify SDA, i.e. the effects of starvation and feeding on the metabolic rate of *B. neritina* colonies (Rubner 1902), (ii) estimate the scaling relationship between metabolic rate and colony size, and (iii) investigate the effects of size manipulation, feeding, and starvation on estimates of metabolic scaling.

Phenotypic plasticity in metabolic rate in response to changing environmental conditions is widespread across taxa and often observed in the field (reviewed in Norin and Metcalfe 2019). Hence, it is reasonable to expect that the ability of an organism to respond to changing conditions by changing its metabolic phenotype should confer a fitness advantage and, therefore, be adaptive. Nevertheless, phenotypic plasticity can only be regarded as being adaptive if organisms respond to environmental change by expressing the phenotype that is in the same direction as the optimal value favoured by selection in the new environment (Pigliucci 2001, Ghalambor et al. 2007). To date, only few studies have formally estimated metabolic plasticity combined with estimates of phenotypic selection on metabolic rate under different environmental conditions in the wild (Pettersen et al. 2018). Furthermore, most studies necessarily rely on fitness proxies such as growth rate or survival rather than the reproductive output of an individual. Nevertheless, estimates of fitness that include reproductive outputs are more likely to fully characterise selection (Pettersen et al. 2018). Thus, the adaptive significance of metabolic plasticity remains largely unknown. Therefore, the aims of Chapter 3 were to (i) examine phenotypic selection on metabolic rate in a benign and a harsh environment, in which *B. neritina* naturally occurs in the field, and (ii) estimate metabolic plasticity in response to a shift from the benign to the harsh environment – combined, these estimates allowed me to provide one of the first formal estimates of the adaptive significance of metabolic plasticity in the field.

The consequences of variation in metabolic rate for population-level processes

Given that metabolic rate is tightly linked to an individual's resource demands (Brown et al. 2004, Burton et al. 2011), it is reasonable to expect that it should scale up to affect processes at higher levels of organization, from populations to communities and ecosystems (Brown et al. 2004). For example, at the population level, metabolic rate is thought to determine a population's carrying capacity – given that larger species usually have higher *per capita* metabolic rates, for a given amount of resources, populations consisting of larger individuals should have lower carrying capacities due to higher *per capita* resource demands (Damuth 1981, Calder 1984, Damuth 1987, Brown et al. 2004, Isaac et al. 2012). Also known as the 'energy equivalence rule', these predictions come mostly from among-species comparisons (Damuth 1981, 1987, Hatton et al. 2015, Perkins et al. 2019), but have rarely been tested directly by keeping body size constant and varying metabolic rates among populations of the same species (but see Bernhardt et al. 2018). Manipulations of metabolic rate independent of body size are important given that body size is correlated with a number of other life-history traits (Calder 1984). Thus, predicted body size – demography relationships may be driven by metabolic rate, but they could also be driven by potentially confounding factors (Tilman et al. 2004).

In contrast to population-level predictions, metabolic theories focussing at energy acquisition and use at the individual level make conflicting predictions (Burton et al. 2011). For example, the 'increased-intake' hypothesis predicts that individuals with higher metabolic rates may be able to feed more voraciously or effectively due to their faster physiologies, such that they can take up or extract more resources from the environment (McNab 1980, Chappell et al. 2007, Biro and Stamps 2010). If and how predictions at the individual level can scale up to affect population-level processes, however, remains unclear.

Thus, in Chapter 4, I provide the first direct test of competing metabolic theories by creating experimental populations of differing densities with different *per capita* metabolic demands. Specifically, I followed the performance (i.e. survival, growth, and reproductive outputs) of individuals within each population throughout their life history in the field to estimate how metabolic rate and population density affect the biomass yield and reproductive outputs of whole populations.

Intraspecific variation in metabolic rate and resource competition

Within a population, individuals compete with other conspecifics for resources – such competitive interactions impose a key constraint for the acquisition of energy by individuals and, consequently, their growth and fitness (Antonovics and Levin 1980, Sinclair et al. 1985, Jenkins et al. 1999, Violle et al. 2010). Given that metabolic rate determines an individual's resource demands (Brown et al. 2004, Burton et al. 2011) and conspecifics usually occupy the same or at least very similar niches, intraspecific competition for resources can exert powerful selective forces on metabolic phenotypes within a population (Pettersen et al. 2020). Here, individuals with higher metabolic rates are often thought to be competitively superior due to their higher activity levels combined with greater boldness, competitive dominance, and territorial aggression (Careau et al. 2008, Biro and Stamps 2010). Similarly, as mentioned above, individuals with higher metabolic rates are thought to feed more voraciously or effectively, such that they may be able to gain preferential access to resources or extract disproportionately more resources from their environment (Pettersen et al. 2020), resulting in asymmetric competition (Weiner 1990). To date, studies investigating competitive interactions among conspecifics of varying metabolic phenotypes have mostly been conducted under laboratory studies – their findings, however, often do not match competitive outcomes in the field (Höjesjö et al. 2002, Auer et al. 2020), although such field

test are rare. In Chapter 5, I therefore tested for the effects of metabolic rate on competitive interactions among *B. neritina* colonies under field conditions. Specifically, I used a trait-specific, response-surface design to create pairwise interactions of individuals with differing metabolic rates (Inouye 2001, Cameron and Marshall 2019). I then measured the outcomes of these interactions by comparing the performance (survival, growth, and reproduction) of individuals with differing metabolic phenotypes, both in the presence and absence of a neighbour colony.

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Chapter 2

Influence of food, body size, and fragmentation on metabolic rate in a sessile marine invertebrate

Abstract

Metabolic rates vary among individuals according to food availability and phenotype, most notably body size. Disentangling size from other factors (e.g., age, reproductive status) can be difficult in some groups, but modular organisms may provide an opportunity for manipulating size experimentally. While modular organisms are increasingly used to understand metabolic scaling, the potential of feeding to alter metabolic scaling has not been explored in this group. Here, I perform a series of experiments to examine the drivers of metabolic rate in a modular marine invertebrate, the bryozoan *Bugula neritina*. I manipulated size and examined metabolic rate in either fed or starved individuals to test for interactions between size manipulation and food availability. Field collected colonies of unknown age showed isometric metabolic scaling, but those colonies in which size was manipulated showed allometric scaling. To further disentangle age effects from size effects, I measured metabolic rate of individuals of known age and again found allometric scaling. Metabolic rate strongly depended on access to food: starvation decreased metabolic rate by 20% and feeding increased metabolic rate by 43%. In comparison to other marine invertebrates, however, the increase in metabolic rate, as well as the duration of the increase (known as specific dynamic action, SDA), were both low. Importantly, neither starvation nor feeding altered the metabolic scaling of my colonies. Overall, I found that field-collected individuals showed isometric metabolic scaling, whereas metabolic rate of size-manipulated colonies scaled allometrically with body size. Thus, metabolic scaling is affected by size manipulation but not feeding in this colonial marine invertebrate.

Introduction

Metabolic rate is a useful indicator of the pace of life, potentially driving ecological and evolutionary processes at all levels of organization (Allen et al. 2005, Brown et al. 2004, Loreau 2010, Sibly et al. 2012, West and Brown 2005, but see Glazier 2015). The rate at which organisms transform and use energy varies extensively among species, populations, and individuals of the same species (Burton et al. 2011, Konarzewski and Książek 2013, White and Kearney 2013). Some levels of this variation in metabolic rate are better understood than others. For example, ectotherms tend to have lower metabolic rates (after accounting for differences in mass) than endotherms, and larger species tend to have lower mass-specific metabolic rates than smaller species (White and Kearney 2013). Likewise, body mass is a strong predictor of metabolic rate within species (Brown et al. 2004, Huxley 1932). Among individuals of the same size and living at the same temperature, however, variation in metabolic rate is less well understood (Burton et al. 2011, Konarzewski and Książek 2013).

Organisms obtain energy from feeding, and food availability can therefore be considered a key factor affecting the life history of an individual (Stearns 1992). Metabolic rate is highly affected by feeding, with individuals typically experiencing a rapid increase in metabolic rate after feeding. Upon reaching peak metabolism, metabolic rate decreases again to pre-feeding levels. This postprandial increase in metabolic rate is termed specific dynamic action (SDA; Rubner 1902) and is significantly influenced by various factors including body size and temperature, and meal size, type, and composition (reviewed in Secor 2009). In recent studies, SDA was found to contribute significantly to an animal's energy budget (McCue 2006, McCue et al. 2005, McCue and Lillywhite 2002, Secor 2009), with observed metabolic rates exceeding maximal metabolism during activity in some species (Andrade et al. 1997, Secor and Diamond 1997, Secor et al. 2000). Specifically, the factorial scope during

SDA, which describes the magnitude of increase in metabolic rate after feeding (Secor 2009), might therefore be a good indicator of the ability of an individual to alter its metabolic rate with changing feeding conditions (i.e. its phenotypic plasticity).

Intuitively, the ability of an organism to alter its metabolic rate with varying food availabilities might be particularly important for sessile invertebrates such as corals, mussels, and bryozoans, as these animals cannot escape rapidly changing environments. To date, few studies have investigated the factorial scope and the overall SDA response of sessile marine invertebrates, and even fewer studies have addressed these responses in colonial marine invertebrates (see Table S1). The factorial scope reported for these species ranges 1.38–6.55 (mean ± SE factorial scope across all marine sessile species: 2.44 ± 0.6, n = 8; Table S1), with observed SDA durations of between 10 and 384 h (mean ± SE: 153.96 ± 68.85 h, n = 5; Table S1). In comparison, across all marine non-sessile invertebrate studies, including semiaquatic species such as crabs, the factorial scope ranges 1.14–5.2 (mean ± SE: 2.25 ± 0.09, n = 76; Table S1). The durations of the SDA response in motile marine invertebrates ranges 4–1200 h (mean ± SE duration across all marine motile invertebrates: 103.49 ± 36.24 h, n = 41; Table S1). Interestingly, to date the only colonial organism for which the metabolic response after feeding was characterized is the coral *Astrangia danae* (factorial scope 1.87; Szmant-Froelich and Pilson 1984).

Metabolic theories seek to understand and predict biological processes at all levels from individuals to populations, communities, and ecosystems (Brown et al. 2004, Nisbet et al. 2000, van der Meer 2006). The relationship between metabolic rate (*MR*) and body mass (*M*) is one of the most studied in biology, particularly since metabolic rate scales allometrically with body mass in most species according to the power function $MR = aM^b$, where a and b are scaling constants and where the fitted value of b is typically less than 1 (e.g., Burgess et al. 2017, Kleiber 1932, Savage et al. 2004). Metabolic scaling provides an

important insight into differences in metabolic efficiencies associated with body mass. Here, metabolic scaling might also be affected by food availability, indicating differences in metabolic efficiencies of different-sized individuals under varying feeding conditions. To date, no studies have formally investigated metabolic scaling in modular organisms under different feeding conditions. In ectotherms more generally, larger individuals typically exhibit a greater SDA response (e.g., Katersky et al. 2006, Luo and Xie 2008, Secor and Faulkner 2002), which often results in steeper metabolic scaling approaching isometry in fed individuals (Secor 2009). Glazier (2010) suggested that near isometric scaling of metabolic rate during SDA arises from the strong influence of volume-related, SDA-induced metabolic demand. In other species, however, no effect of body size was found (e.g., Boyce and Clarke 1997, Grigoriou and Richardson 2008). In colonial organisms (i.e. clonal organisms that are subdivided into functionally autonomous or semi-autonomous, multicellular modules; Vuorisalo and Tuomi 1986), feeding differs from that in unitary organisms in the way that colonial animals take up and transform resources. Most studies indicate that feeding rates scale isometrically or superlinearly in colonial animals (e.g., Okamura 1984, 1985, Pratt 2005), whereas metabolic scaling is allometric in most species (Barneche et al. 2017, Burgess et al. 2017, Hartikainen et al. 2014). Hence, as colonies increase in size, their capacity to capture food increases more quickly than the rate at which they expend energy. Under varying feeding conditions, colonies have been shown to shut down the number of actively feeding modules in response to extremely low or high food concentrations (Riisgård and Larsen 2000). Thus, changes in metabolic scaling with varying feeding conditions can be anticipated as the number of actively feeding modules changes disproportionally across colony sizes. For example, when food is restricted, all modules in smaller colonies might be actively feeding, while only a small number of modules are active in larger colonies. These differences in the number of feeding modules across colony sizes, in turn, may result in a

decrease in metabolic rates in larger colonies but not in smaller ones, constituting an overall shallower metabolic scaling under restricted feeding conditions. Whether metabolic scaling in colonial organisms varies with changing feeding conditions according to these hypotheses remains to be studied.

Most studies investigating the scaling relationship between metabolic rate and body mass must rely on natural variation in body size among individuals, and those investigations are potentially confounded by the effect of other traits such as age and nutrition that covary with body size (e.g., Calder 1984, Schmidt-Nielsen 1984). Colonial animals provide an opportunity to manipulate size independently of other factors, thereby minimizing potentially confounding effects (e.g., those associated with age). Size manipulation approaches in several recent studies show how size can be manipulated to test predictions of competing theories (Barneche et al. 2017, Nakaya et al. 2005, White et al. 2011, reviewed in Burgess et al. 2017).

Three types of scaling relationship between metabolic rate and body mass have been recognized: ontogenetic, static, and evolutionary scaling (e.g., Cheverud 1982, Pélabon et al. 2013, White and Kearney 2014). Ontogenetic scaling considers the relationship between metabolic rate and mass in the same individual through developmental time (e.g., Killen et al. 2007). Static scaling considers the relationship between metabolic rate and mass among individuals of the same developmental stage within a species (e.g., Pettersen et al. 2015). Evolutionary scaling considers the relationship between metabolic rate and mass among individuals of different species, again at the same developmental stage (e.g., Savage et al. 2004). Distinguishing between these forms of metabolic scaling is particularly important when comparing models that have been proposed to explain scaling relationships (see White and Kearney 2014). The main objectives of the present study are to investigate how size affects metabolic rate (i.e., metabolic scaling) in the marine colonial bryozoan *Bugula*

neritina, and how size manipulations, feeding, and starvation affect estimates of metabolic scaling.

Materials and methods

Study species, size manipulation, and measurement of metabolic rate

Bugula neritina Linnaeus 1758 is a colonial, arborescent bryozoan commonly found as part of the fouling community on artificial structures throughout the world. Adult colonies of *B. neritina* grow via asexual budding by producing new pairs of zooids (individual subunits) at the distal ends of the branches of the colony. Regular bifurcations give the colony an arborescent shape (Keough 1989, Keough and Chernoff 1987). Once colonies are sexually mature, zooids develop clearly visible brood structures known as ovicells, in which offspring are brooded (Woollacott and Zimmer 1975).

I collected non-reproductive colonies of *B. neritina* from the Royal Brighton Yacht Club in Port Phillip Bay, Vic., Australia (−37.909, 144.986), between August and November 2017. I transported colonies to the laboratory and maintained them in aerated tanks in field-collected seawater at 19°C for up to 12 h prior to their use in experiments.

Because of the colonial nature of *B. neritina*, I was able to manipulate the size of non-reproductive colonies by cutting off the basal part of the colony, thereby creating two different treatments: size-manipulated and intact colonies (Fig. 1). I measured colony size as the number of bifurcations, and size-manipulated colonies were derived from bigger colonies (e.g., I cut off the lower part of a colony that was six bifurcations in size to derive a size-manipulated colony that was five bifurcations in size). By doing so, size-manipulated and intact colonies used for experiments were of comparable size. Size-manipulated colonies were allowed to recover for 1 h before measurements.

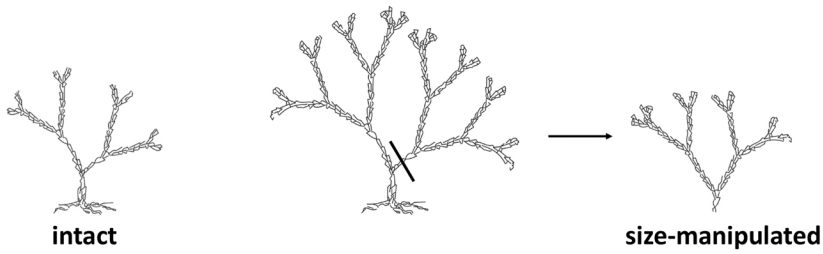

Figure 1: Schematic illustration of my fragmentation approach, allowing me to investigate the effect of size manipulation on the Routine metabolic rate and metabolic scaling in *Bugula neritina*. Importantly, by applying this size manipulation approach, intact and size-manipulated colonies were of comparable size, and the overall colony form was maintained.

Based on comparisons of the average age of zooids in the size-manipulated and intact colonies, I found that my size manipulation approach did not alter the covariance between body size and age substantially and was, therefore, an appropriate test of the effects of size manipulation on metabolic rate in this species. Importantly, and in contrast with the size manipulation approach recently applied for *B. neritina* by Barneche et al. (2017), the overall colony form was maintained in both treatments (for comparison, see Fig. 1 and Barneche et al. 2017: Fig. 1).

To determine metabolic rate, I measured the oxygen consumption rate ($\dot{V}O_2$, a commonly used proxy for metabolic rate) of individual colonies. Due to the presence of spontaneous activity such as expanding and retracting of the feeding structures during measurements, I defined metabolic rate as Routine MR (Mathot and Dingemanse 2015). Before measurements of metabolic rate, I inspected colonies for epibionts such as amphipods and ciliates. I carefully removed any epibionts with a forceps and cleaned each colony using a soft-tipped paint brush. I placed individual colonies into 5-ml SDR glass vials (PreSens, Germany) containing sterilized, 0.2-µm filtered seawater (FSW) and a non-consumptive O_2 sensor spot. To prevent colonies from touching the sensor spot during measurements, I placed

a small sheet of acetate diagonally between the sensor spot and the colony. Measurements of $\dot{V}O_2$ were conducted using 24-channel PreSens sensor dish readers (Sensor Dish Reader SDR2, PreSens, Germany), along with four controls (blank vials containing only seawater and acetate) per SDR reader. Prior to $\dot{V}O_2$ measurements, I calibrated the sensor spots with air-saturated (AS) seawater (100% AS) and seawater containing 2% sodium sulfite (0% AS). Measurements of $\dot{V}O_2$ were recorded in a darkened, constant-temperature room at 19°C over 3 h (for an example of the SDR outcome, refer to Fig. S1).

I used the R package *LoLinR* (Olito et al. 2017), which implements local linear regression techniques for estimating monotonic biological rates from time-series or trace data, to determine the optimal measurement interval (i.e., the most linear part of the measurement curve). I excluded the first 30 min of the 3-h measurement period (during which colonies might show oxygen consumption patterns that reflect recovery from handling procedures). As *LoLinR* only considers a fraction of the measurement curve, I additionally ran the program using different parts of the curve, thus ensuring that I did not miss peak $\dot{V}O_2$. I used either the first half or the last half of the curve (after excluding the first 30 min of the overall measurement time) and compared the output to the results obtained from analysis of the whole measurement curve. Routine MR values calculated from the last half and the overall curve were very similar, but metabolic rates calculated from the first half of the curve were slightly lower (Fig. S2). Those slightly lower MR values could be attributed to a delayed detection of small decreases in oxygen in the SDR vials, especially in measurements from smaller colonies. Nevertheless, these findings indicate that I indeed captured the maximum peak $\dot{V}O_2$ of *B. neritina*.

Using the *LoLinR* output, I calculated $\dot{V}O_2$ from the rate of change of O_2 saturation over time (m_a; in % per hour) as per White et al. (2011):

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

where m_b is the rate of change of O_2 saturation for control vials (% per hour), βO_2 is the oxygen capacitance of air-saturated seawater at 19°C (5.31 ml/L; Cameron 1986), and V is the water volume (the volume of the animals was subtracted from the total vial volume of 5 ml). To convert $\dot{V}O_2$ (ml per hour) to metabolic rate (milliJoules per hour), I used the calorific conversion factor of 20.08 J/ml O_2 (Crisp 1971).

Ontogenetic versus static metabolic scaling (Experiment 1)

Barneche et al. (2017) have recently shown that metabolic rate scales with body size with an allometric scaling exponent of 0.72 in size-manipulated colonies of *B. neritina* at a slightly higher temperature (25°C) than the one I used in the present study (19°C). When collecting colonies of varying body sizes from the field, however, the ontogenetic stages of these colonies were unknown. Therefore, in order to estimate the contribution of ontogenetic variation to metabolic scaling in *B. neritina*, I conducted a field experiment in which I investigated and compared the ontogenetic and static scaling of metabolic rate in *B. neritina*.

I collected reproductive colonies of *B. neritina* at the Royal Brighton Yacht Club in November 2017 and induced spawning according to standard light-shock procedures: colonies were kept in darkened, aerated tanks at 19°C for 48 h and then placed in beakers filled with seawater and are exposed to bright light (Marshall et al. 2003). I then pipetted the released larvae in a drop of seawater directly onto two roughened A4 acetate sheets to induce settlement. Following settlement, I randomly assigned the acetate sheets to one of two PVC backing panels (570 × 570 × 6 mm), and suspended these panels 1 m below the water surface in a horizontal orientation, with the newly settled colonies on the underside of the panel facing the substrate, at the Royal Brighton Yacht Club (for a detailed description of the field deployment, see Marshall and Keough 2009). After 3 weeks in the field, I brought the acetate sheets with colonies back to the laboratory to conduct metabolic rate measurements (I refer to

these initial data as Measurement 1; see Section 2.1, above). I also counted the number of zooids and ovicells in each colony as a proxy for body mass, to avoid the inaccuracies inherent in weighing colonies attached to the acetate sheets. In *B. neritina*, the number of zooids and body mass are highly correlated ($r_{33} = 0.91$, $p < 0.0001$; Fig. S3). Following measurements, I glued individual colonies back onto smaller squares of acetate sheet (55 × 55 mm), assigned each colony an identifying number, and deployed the colonies in the field again to allow them to grow. I then conducted further metabolic rate measurements and counted the number of zooids and ovicells 1 week (Measurement 2), 2 weeks (Measurement 3), and 3 weeks (Measurement 4) following the first measurement.

Effects of starvation and size manipulation on Routine MR (Experiment 2a)

To investigate the effect of starvation and size manipulation on the Routine MR of *B. neritina*, I collected 60 colonies and assigned them to a size-manipulated treatment (n = 29) or an intact colony treatment (n = 31). I then acclimated the colonies in field-collected seawater at 19°C in a constant-temperature room for 3 h prior to the initial metabolic rate measurement. Following the first measurement, I estimated colony mass by blotting dry and weighing each colony to the nearest 0.01 g. I then incubated all colonies in 15 L of FSW in a big cooler box, with each colony placed inside a 60-ml glass jar completely submerged in the FSW. I then conducted metabolic rate measurements 24, 36, and 48 h following the initial metabolic rate measurement. I renewed the FSW daily.

Disentangling the effects of starvation and laboratory conditions on Routine MR (Experiment 2b)

To test whether the Routine MR of colonies kept in the laboratory decreased as a response to starvation or to laboratory conditions more generally, I used a total of 35 colonies that were

assigned to a fed treatment (n = 16) or a starved treatment (n = 19). Within the fed treatment, eight colonies were size manipulated, and eight colonies were left intact. Within the starved treatment, nine colonies were size manipulated, and 10 colonies were intact. As in Experiment 1, I acclimated freshly collected colonies in field-collected seawater at 19°C for 3 h prior to the first metabolic rate measurement. Subsequently, I weighed each colony and incubated colonies in the starved treatment in 60-ml glass jars containing 50 ml of FSW. Colonies in the fed treatment were incubated in 60-ml glass jars containing 50 ml of unfiltered seawater to which I added the green alga *Dunaliella tertiolecta* (Butcher; Australian National Algae Culture Collection; strain code CS-14) at a concentration of 10,000 cells/ml (based on measurements of optical density). I chose this alga because a previous cultivation study by Kitamura and Hirayama (1984) and my unpublished pilot studies showed that colonies of *B. neritina* consume and grow on this diet in the laboratory. I conducted metabolic rate measurements of colonies 24, 48, 72, 96, 120, and 144 h after the initial metabolic rate measurement. As in Experiment 1, I renewed the seawater and added algae to the colonies in the fed treatment after each metabolic rate measurement.

Effect of size on specific dynamic action (Experiment 3)

To determine the effect of body size and size manipulation on the specific dynamic action (SDA) of *B. neritina*, I collected 56 colonies and assigned them to a size-manipulated treatment (n = 29) or an intact colony treatment (n = 27). I then incubated colonies in 15 L of FSW (each colony was placed in a 60-ml glass jar, as described in Experiment 1, for 24 h at a temperature of 19°C. Following an initial metabolic rate measurement to determine the baseline metabolism of starved colonies, I weighed each colony, returned them to seawater, and added the red alga *Rhodomonas salina* (Australian National Algae Culture Collection; strain code CS-692) to 15 L of FSW to approximate a concentration of 10,000 cells/ml (based

on measurements of optical density). I chose this algal concentration because it supports the highest growth rates in colonies of *B. neritina* (based on Kitamura and Hirayama 1984 and unpublished pilot studies). After adding algae, I collected water samples and fixed algae in a 2% Lugol solution. I then estimated the phytoplankton concentration by manual cell counts (using a Neubauer hemocytometer). The average phytoplankton concentration was ~11500 cells/ml. Colonies were fed for a total of 4 h, but I completely renewed the FSW supplemented with algae after 2 h. Following feeding, I rinsed all colonies in FSW for 1 h to remove excess algae, and then determined the peak $\dot{V}O_2$ of each colony. I then kept colonies in 15 L of FSW and conducted further metabolic rate measurements 16 and 24 h after feeding. I used these data to quantify the factorial scope of peak $\dot{V}O_2$ (calculated as peak $\dot{V}O_2$ divided by the baseline metabolism), based on the mean Routine MR of colonies before and after feeding, as described by Secor (2009).

Statistical analyses

For statistical analyses, I used a repeated measures design analysis of co-variance (ANCOVA), with treatment (size-manipulated vs. intact colonies), feeding (fed vs. starved colonies), time (metabolic rate measurement points) and the number of zooids (Experiment 1) or colony mass (Experiments 2a,b and Experiment 3), and all their possible interactions as fixed effects and as a covariate. Routine MR and the number of zooids or mass were log transformed prior to analyses. I included colony ID nested within mass as a random factor. Model reduction was conducted by removing nonsignificant interactions if their inclusion did not improve the model fit (Quinn and Keough 2002). I further conducted Wald tests for differences in the scaling exponents. Scaling exponents were derived using a log-transformed linear relationship as $\log_{10}(\text{Routine MR}) = b \times \log_{10}(\text{Mass}) + \log_{10}(a)$ (Experiments 2a,b and Experiment 3), or $\log_{10}(\text{Routine MR}) = b \times \log_{10}(\text{Zooids}) \times \log_{10}(\text{Ovicells}) + \log_{10}(a)$

Table 1: Summary of scaling exponents (b) (±SE) and coefficients (a) for metabolic rate and mass (intact and size-manipulated colonies) or the number of zooids (ontogenetic and static scaling) in colonies of *Bugula neritina*, using a log-transformed linear relationship between metabolic rate and mass or number of zooids (see Section 2).

			Scaling				
		Coefficient	exponent	*P*-value	*P*-value	*P*-value	
	n	**(*a*)**	**(*b*) ± SE**	***b* ≠ 0**	***b* ≠ 1**	***b* ≠ 0.75**	R^2
Intact colonies	31	0.62	0.96 (± 0.079)	**<0.0001**	0.66	**<0.05**	0.84
Size-manipulated colonies	29	0.86	0.71 (± 0.071)	**<0.0001**	**<0.001**	0.58	0.79
Ontogenetic scaling	260	-0.97	1.04 (± 0.05)	**<0.0001**	0.48	**<0.0001**	0.82
Static scaling, 3 weeks old	65	-0.49	0.83 (± 0.1)	**<0.0001**	0.1	0.46	0.5
Static scaling, 4 weeks old	65	-0.61	0.93 (± 0.09)	**<0.0001**	0.42	0.06	0.61
Static scaling, 5 weeks old	65	-0.12	0.77 (± 0.12)	**<0.0001**	0.06	0.85	0.4
Static scaling, 6 weeks old	65	-0.1	0.81 (± 0.08)	**<0.0001**	**<0.05**	0.46	0.61

(Experiment 1), respectively, if colonies were reproductive. In *B. neritina*, the number of zooids within a colony is linearly related to colony mass (Fig. S3). Furthermore, scaling exponents were similar across experiments when using either the number of zooids or mass in regression analyses. All statistical analyses were conducted in R (R Core Team 2017) using the package *lmerTest* (Kuznetsova et al. 2017).

Results

Ontogenetic versus static metabolic scaling (Experiment 1)

My estimates of ontogenetic and static scaling differed in field-collected colonies of *Bugula neritina*. Although metabolic rate throughout ontogeny scaled isometrically with the number of zooids at an exponent of 1.04, static scaling exponents ranged between 0.77 (Measurement 3) and 0.93 (Measurement 2). Static scaling exponents were not significantly different from 0.75 (Table 1; Fig. 2).

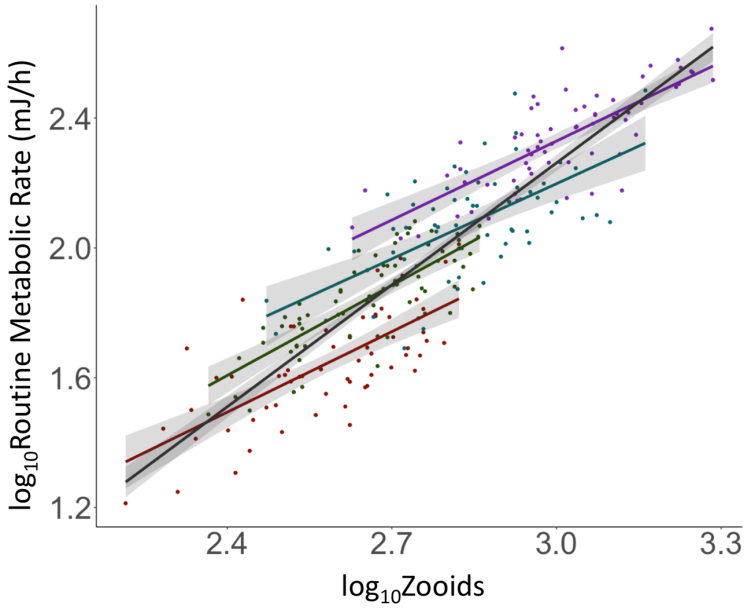

Figure 2: Relationship (log-transformed) between routine metabolic rate (mJ/h) and the number of zooids within a colony in *Bugula neritina*. The black line indicates the ontogenetic scaling relationship across developmental stages, while the coloured lines indicate the static scaling at different developmental stages. Regression lines indicate scaling in colonies that are 3 weeks (red line), 4 weeks (green line), 5 weeks (blue line), or 6 weeks old (purple line). Regression lines were derived using a log-transformed linear relationship, where $\log_{10}(\text{Routine MR}) = b \times \log_{10}(\text{Zooids}) \times \log_{10}(\text{Ovicells}) + \log_{10}(a)$; a and b are scaling exponents; "Zooids" and "Ovicells" refer to the number of zooids and ovicells, respectively, in a colony. Data points represent single colonies repeatedly measured over time. Grey areas indicate the 95% confidence intervals. Scaling exponents are presented in Table 1.

Effects of starvation and size manipulation on Routine MR (Experiment 2a)

I found an interaction between the size manipulation treatment and body mass, with larger size-manipulated colonies having a lower Routine MR than larger intact colonies (Table 2; Fig. 3). The scaling exponent for intact colonies was b = 0.96 and did not differ significantly from 1, whereas size-manipulated colonies scaled at b = 0.71. The scaling exponent for size-manipulated colonies differed significantly from 1 (Table 1), and also differed significantly

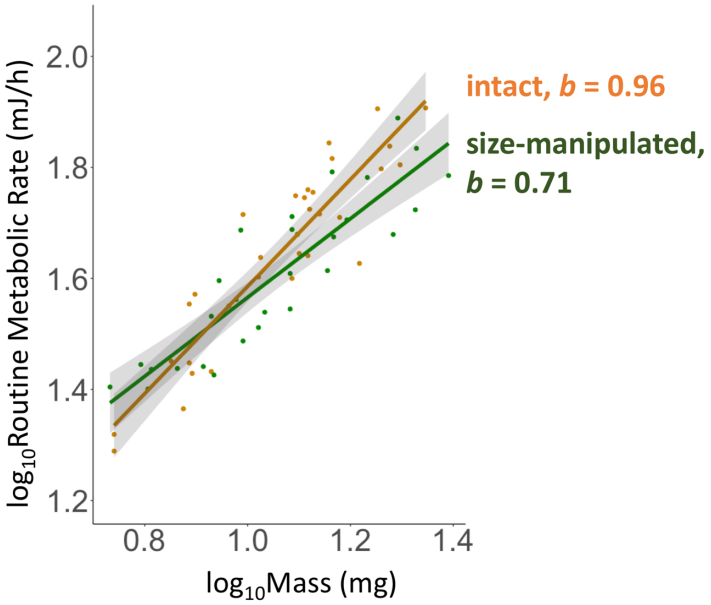

Figure 3: Relationship (log-transformed) between routine metabolic rate (mJ/h) and mass (mg) for freshly collected colonies of *Bugula neritina*. The green line indicates the scaling relationship of size-manipulated colonies, the yellow line indicates the scaling relationship of intact colonies. Regression lines were derived using a log-transformed linear relationship, where $\log_{10}(\text{Routine MR}) = b \times \log_{10}(\text{Mass}) + \log_{10}(a)$. Each data point represents a single colony. Grey areas indicate the 95% confidence intervals.

from the scaling exponent of intact colonies (Wald test; $P < 0.01$).

During starvation, the Routine MR in colonies of *B. neritina* decreased overall (Fig. 4), and although the effect of colony mass was consistent, the effect of size manipulation changed over time (Table 2). Intact colonies had a higher mean Routine MR than size-manipulated colonies at time point T0 (freshly collected colonies). After 24 h, however, intact and size-manipulated colonies had reached similar mean Routine MRs, and metabolic rates decreased at similar rates in both treatments.

Table 2: Repeated measures ANCOVA examining the effects of mass and treatment (size-manipulated vs. intact) on the Routine metabolic rate in colonies of *Bugula neritina* during starvation. Nonsignificant interactions were removed from the final model (Table S2).

	df	**F**	***P***
Between subjects			
$\log_{10}$(Mass)	1	462.692	**<0.0001**
Treatment	1	7.906	**0.007**
$\log_{10}$(Mass) × treatment	1	8.247	**0.006**
Error	56		
Within subjects			
Time	3	159.776	**<0.0001**
Treatment × time	3	3.214	**0.024**
Error	174		

Disentangling the effects of starvation and laboratory conditions on Routine MR (Experiment 2b)

I found that feeding (starved vs. fed) had a significant effect on the Routine MR of colonies over time (Fig. 5; Table 3). Although the Routine MR of starved colonies decreased by 20%, the Routine MR of fed colonies remained constant over time. There was a significant interaction between mass and time (Table 3): the scaling exponent of the relationship between mass and Routine MR of both starved and fed colonies decreased over time. Notably, the interaction between mass and feeding was not significant, indicating that metabolic scaling was not significantly different between starved and fed colonies ($F_{1,31}$ = 2.303, P = 0.141; Table S3), but there was a trend for starved colonies to have lower metabolic scaling exponents (Table 3). I did not detect any significant difference between size-manipulated and intact colonies, and the interaction between treatment and time was nonsignificant (Table 3). Therefore, for visualization purposes, I plotted metabolic scaling regression lines for both treatments combined (Fig. 5).

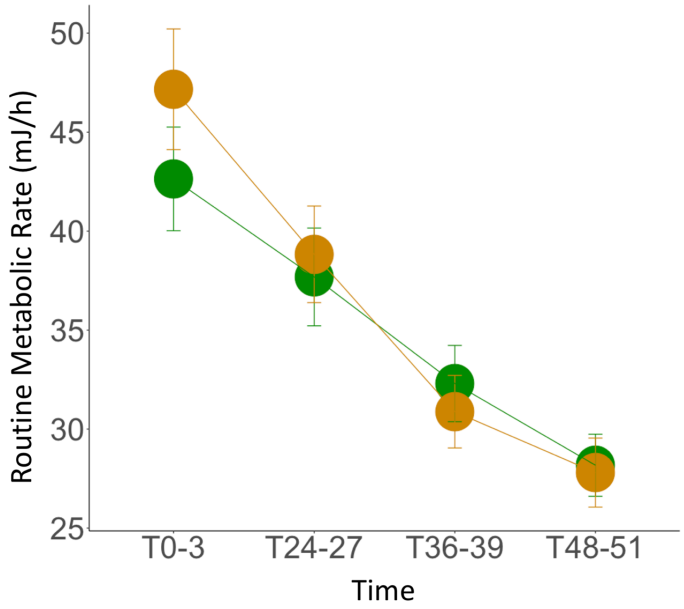

Figure 4: The change in mean routine metabolic rate (mJ/h) of starved colonies of *Bugula neritina* over time. Orange dots indicate the decrease in mean metabolic rate over time of intact colonies. Green dots indicate the decrease in mean metabolic rate over time of size-manipulated colonies. T0-3 depicts the mean metabolic rate of freshly collected colonies, T24-27, T36-39, and T48-51 represent the mean metabolic rate of starved colonies after 24–27, 36–39, and 48–51 h, respectively. Error bars indicate the standard error.

Effect of size on specific dynamic action (Experiment 3)

The Routine MR in colonies of *B. neritina* increased significantly after feeding (factorial scope = 1.43; Fig. 6; Table 4). After 16 h, Routine MR was still elevated, but colonies reverted to their pre-feeding Routine MR 24 h after feeding (Fig. 7). Interestingly, the scaling relationship between mass and Routine MR did not change over time (Table 4), indicating that the SDA response was similar across all body sizes. Also, the effect of size manipulation did not change over time (Table 4). Size-manipulated colonies, however, had on average a higher Routine MR than intact colonies (Fig. 7).

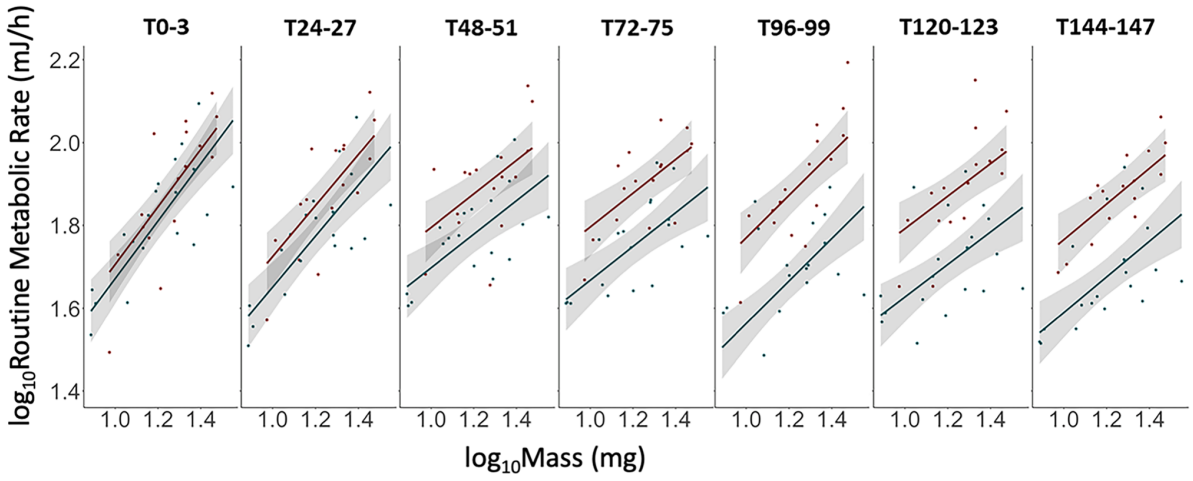

Figure 5: Predicted relationships (log-transformed) between routine metabolic rate (mJ/h) and mass (mg) of starved (blue lines) and fed (red lines) colonies of *Bugula neritina* over time. T0-3 depicts the metabolic rate of freshly collected colonies. T24-27, T48-51, T72-75, T96-99, T120-123, and T144-147 represent the metabolic rate of starved (blue points and lines) and fed (red points and lines) colonies after 24–27, 48–51, 72–75, 96–99, 120–123, and 144–147 h, respectively. Regression lines were derived using a log-transformed linear relationship, where $\log_{10}(\text{Routine MR}) = b \times \log_{10}(\text{Mass}) + \log_{10}(a)$. Each data point represents a colony repeatedly measured over time. Grey areas indicate the 95% confidence intervals.

Discussion

Metabolic responses to varying feeding conditions are typically characterized by a decrease in metabolic rate during starvation, and an increase in metabolic rate after the ingestion of a meal (reviewed in Secor 2009). My findings are consistent with previous studies in marine invertebrates, in which metabolic rate changed with varying food availabilities (see Table S1). Under food deprivation, Routine MR decreased by 20% (Experiments 2a,b), and this response was independent of laboratory conditions (Experiment 2b). Upon feeding, metabolic rate increased significantly by 43% and remained elevated for 24 h before returning to pre-

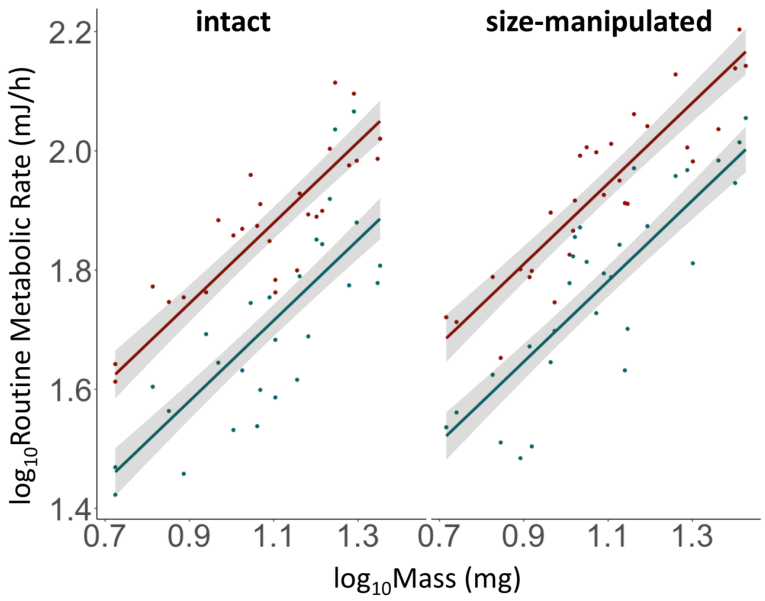

Figure 6: Predicted relationships (log-transformed) between routine metabolic rate (mJ/h) and mass (mg) of intact (left panel) and size-manipulated (right panel) colonies of *Bugula neritina* before (i.e., starved colonies; blue lines) and after feeding (red lines). Each data point represents a colony measured before (blue dots) and after feeding (red dots). Regression lines were derived using a log-transformed linear relationship, where $\log_{10}(\text{Routine MR}) = b \times \log_{10}(\text{Mass}) + \log_{10}(a)$. Grey areas indicate the 95% confidence intervals.

feeding levels (Experiment 3). Size manipulation, furthermore, had unanticipated effects on the metabolic scaling of *B. neritina*. Although ontogenetic scaling is isometric, size manipulation resulted in a shift to allometry (Experiment 1), which corresponds to the static scaling in this species (Experiment 2a).

Food availability can have significant effects on the phenotype of an organism. Palumbi (1984) showed that demosponges change their overall body structure to maximize feeding in highly turbulent environments. Similarly, colonies of *B. neritina* develop smaller feeding structures when growing in environments with high density of conspecifics (Thompson et al. 2015), and in such colonies the individual feeding rates are typically

Table 3: Repeated measures ANCOVA examining the effects of mass, treatment (size-manipulated vs. intact), and feeding (starved vs. fed) on the Routine metabolic rate in colonies of *Bugula neritina*. Nonsignificant interactions were removed from the final model (Table S3).

	df	**F**	***P***
Between subjects			
$\log_{10}$(Mass)	1	51.511	**<0.0001**
Treatment	1	2.735	0.108
Feeding	1	28.536	**<0.0001**
Error	31		
Within subjects			
Time	6	1.991	0.069
$\log_{10}$(Mass) × time	6	2.347	**0.033**
Feeding × time	6	5.728	**<0.0001**
Treatment × time	6	2.022	0.065
Error	186		

decreased (Amundsen et al. 2007, Damuth 1981). In addition to this morphological plasticity, I show that metabolic rate changes with increased or restricted food availability. Importantly, this ability to alter metabolic rate can have implications for the life history of an individual (Stearns 1992). In brown trout, for example, individuals that were best able to adjust metabolic rate had the highest growth rate under changing food availability (Auer et al. 2015). Similarly, growth rate is positively correlated with the magnitude of the factorial scope during SDA in the common starfish, *Asterias rubens* (Vahl 1984). Therefore, SDA is often used as an index of the energetic cost of growth or biosynthesis (Kiørboe et al. 1987, Wieser 1994).

In *B. neritina*, Svensson and Marshall (2015) showed that food availability affects colony growth. Similarly, body size and fitness decrease with increasing conspecific densities (Allen et al. 2008, Ghedini et al. 2017, Hart and Marshall 2013). High conspecific densities,

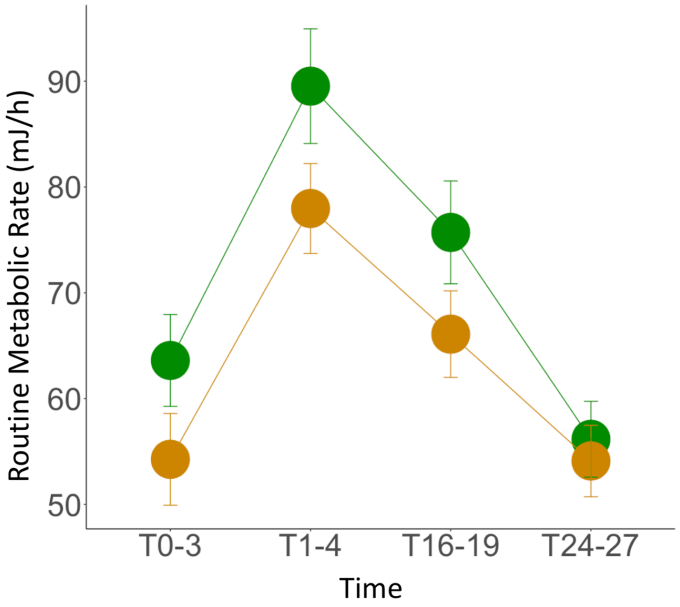

Figure 7: The change in mean routine metabolic rate (mJ/h) of colonies of *Bugula neritina* during specific dynamic action. Orange dots indicate the SDA response of intact colonies. Green dots indicate the SDA response of size-manipulated colonies. T0-3 depicts the mean metabolic rate of colonies that were starved for 24 h prior to measurements. T1-4 represents the mean peak metabolic rate of colonies 1–4 h after feeding. T16-19 and T24-27 are the mean metabolic rates of starved colonies 16–19 and 24–27 h after feeding. Error bars indicate the standard error.

furthermore, result in decreased individual metabolic rates along with decreased feeding rates (Ghedini et al. 2017). Here, reduced oxygen availabilities (Lagos et al. 2017), or the presence of metabolites from conspecifics (Thompson et al. 2015), have been proposed to drive the observed decrease in metabolic rates. My results, however, suggest that reductions in food availability alone (possibly associated with increased density) could drive these changes in metabolic rate.

Both the factorial scope and the duration of the overall SDA response vary among species (Secor 2009). In comparison to other sessile marine invertebrates, I found that *B. neritina* had one of the lowest factorial scopes reported to date and the duration of the overall

Table 4: Repeated measures ANCOVA examining the effects of mass and treatment (size-manipulated vs. intact) on the Routine metabolic rate in colonies of *Bugula neritina* during SDA. Nonsignificant interactions were removed from the final model (Table S4).

	df	**F**	***P***
Between subjects			
$\log_{10}$(Mass)	1	242.607	**<0.0001**
Treatment	1	10.476	**0.002**
Error	53		
Within subjects			
Time	3	119.887	**<0.0001**
Treatment × time	3	2.403	0.069
Error	162		

SDA response was lower than the median duration reported for other sessile marine invertebrates (see Fig. 8). An organism's SDA is affected by various factors including meal type and size, and body size and temperature (Secor 2009). For example, SDA in ectotherms living in colder environments is generally lower and lasts longer than in ectotherms in warmer environments (e.g., Peck and Veal 2001). Although the factorial scope may be similar across all body sizes in some species (e.g., McGaw and Curtis 2013), in most species the factorial scope increases with body size (e.g., Boyce and Clarke 1997). The effects of meal type and size have been studied in several marine invertebrates (e.g., McGaw and Curtis 2013, Rosas et al. 2001). Typically, an animal's factorial scope and duration increase with increased meal sizes and with meal types that are costlier to digest. In *B. neritina*, Kitamura and Hirayama (1984) found that colonies had the highest growth rate when fed with *R. salina* at a concentration similar to the one I used in the present study. Furthermore, when fed at very high algal concentrations, feeding activity in bryozoans decreases because the number of actively feeding zooids is reduced (Riisgård and Goldson 1997). Thus, I am confident that the factorial scope reported here represents the upper limit for the postprandial increase in

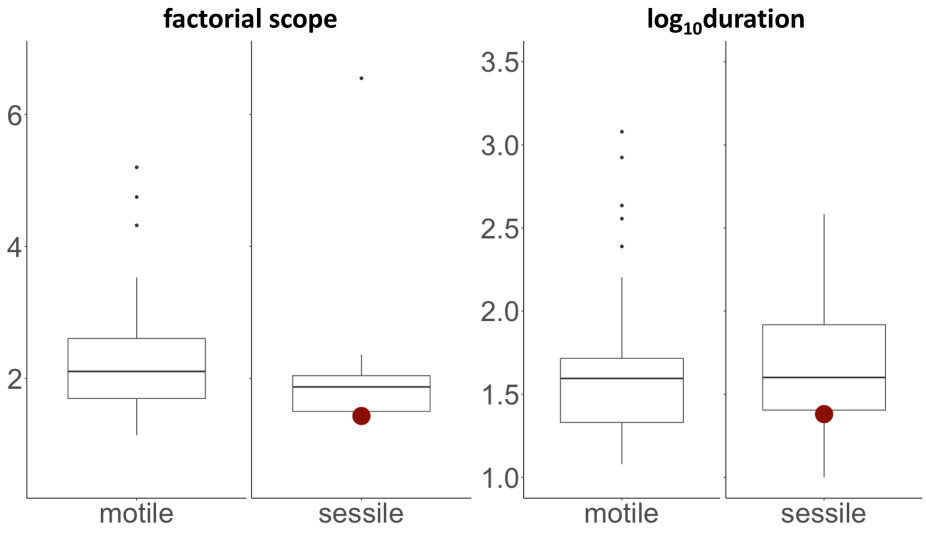

Figure 8: Factorial scopes and durations of specific dynamic action (SDA) in sessile and motile marine invertebrates, respectively (listed in Table S1). The red dot indicates either the scope or the duration of SDA in the colonial bryozoan *Bugula neritina*.

metabolic rate in *B. neritina*. Why the observed factorial scope in *B. neritina* is so low, however, cannot be easily explained by factors such as meal type or size, or body size or temperature.

In comparison with my study, Sigsgaard et al. (2003) found that the ascidian *Ciona intestinalis* exhibited one of the highest factorial scopes across all studied sessile marine invertebrates, and a comparably longer duration. Similarly, other sessile species, including species of molluscs, were found to have greater scopes and longer durations than *B. neritina* when fed with algae at both lower and higher temperatures (see Table S1). As far as I are aware, the only other colonial animal for which SDA has been characterized is the coral *A. danae* (Szmant-Froelich and Pilson 1984). When fed a shrimp meal, colonies of *A. danae* exhibited a slightly higher factorial scope than that of *B. neritina*; the overall duration of SDA, however, was not reported (see Table S1). The factorial scope in *A. danae* was also low relative to other marine invertebrates (fifth lowest overall). Whether modularity (i.e., the fact

that many small subunits take up and transform resources within a colony) is the cause of this relatively modest SDA response remains to be studied.

Modular animals are useful for testing theories of metabolic scaling, as it is possible to manipulate their size and shape (reviewed in Burgess et al. 2017). In a colonial ascidian, Nakaya et al. (2003) found that metabolic scaling switched from allometry to isometry during the takeover stage of the colony, in which the zooids of the parent generation in a colony degenerate and zooids of a new generation develop in unison. Size manipulation, and also fusion of various colonies, however, did not affect the metabolic scaling in this species (Nakaya et al. 2005). White et al. (2011) did not find any differences in the allometric metabolic scaling between intact and size-manipulated colonies of the encrusting bryozoan *Hippoporina indica*. In the arborescent freshwater bryozoan *Fredericella sultana*, Hartikainen et al. (2014) showed that metabolic scaling in size-manipulated colonies is allometric. Similarly, allometric scaling has recently been demonstrated in size-manipulated colonies of *B. neritina* (Barneche et al. 2017). As I show here, metabolic scaling in field-collected intact colonies is isometric rather than allometric. It seems that the differences in metabolic scaling might be driven by unanticipated effects of size manipulation on metabolic rate in size-manipulated colonies. Within cheilostome bryozoans such as *B. neritina*, zooids are connected by pores in the interzooid walls (Best and Thorpe 2001, Bobin 1977, Lutaud 1985, Mukai et al. 1997). Size manipulation might lead to leaking of nutrients, driving the differences in metabolic scaling between size-manipulated and intact colonies. Notably, both the size-manipulation approaches conducted by Barneche et al. (2017) and my approach (refer to Fig. 1) reported similarities in the scaling of size-manipulated colonies. Although in their study, Barneche et al. (2017) cut off the tips of colonies to mimic natural predation, in my study I retained the upper part of the colony and discarded the stolon. As both approaches resulted in the allometric metabolic scaling of fragments, these findings indicate that

variation in colony form is unlikely to drive the observed differences in the scaling. To fully understand the effects of size manipulation on metabolic rates and biological processes within *B. neritina* colonies, further studies are needed.

Overall, I find that neither feeding nor starvation alter metabolic scaling exponents in *B. neritina*. Size manipulation has unanticipated effects on metabolic scaling in this species. Although field-collected individuals of unknown age scale isometrically, metabolic scaling in size-manipulated colonies is allometric, which corresponds with the static scaling of this species. There appears to be an unusually short SDA period and low scope in *B. neritina*. Whether this is a species-specific trait or one driven by coloniality is unclear at this stage, and I encourage further tests of SDA in other colonial marine invertebrates.

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Supplementary material

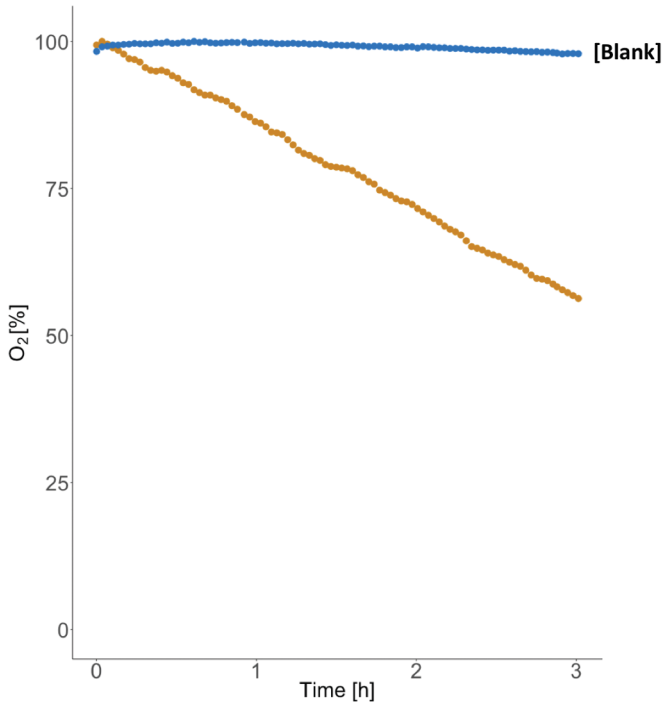

Figure S1: Example of the SDR outcome, where the oxygen concentration in the SDR vials is measured over a time period of 3h. The blue dots are the mean blank representing the mean of four individual blanks per reader. The yellow dots depict the decrease in oxygen concentration for a single colony. Each dot represents a single oxygen concentration measurement, with a measurement interval of 2 mins.

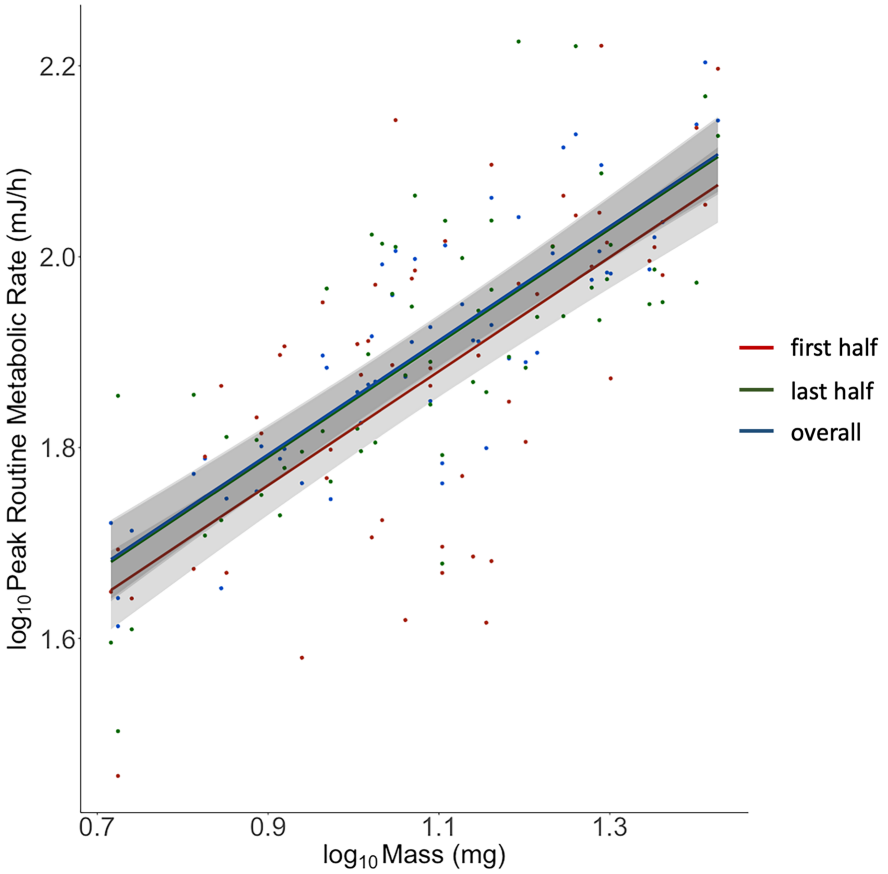

Figure S2: Predicted relationships between (log-transformed) peak routine metabolic rate (mJ/h) and mass (mg) of *Bugula neritina* colonies when implementing the R package *LoLinR* on different parts of the 3h measurement curve. The red line indicates the scaling relationship of metabolic rates calculated from the first half of the measurement curve. The green line indicates the scaling relationship of metabolic rates calculated from the last half of the measurement curve. Finally, the blue line indicates the scaling relationship of metabolic rates calculated from the overall measurement curve. Each data point represents a colony for which either the first half (red dots), the last half (green dots) or the overall measurement curve (blue dots) was used to derive peak metabolic rates using *LoLinR*. Regression lines were derived using a log transformed linear relationship, where $\log_{10}$Routine MR = b × $\log_{10}$Mass + a. Each data point represents a single colony. Grey areas indicate the 95% confidence intervals.

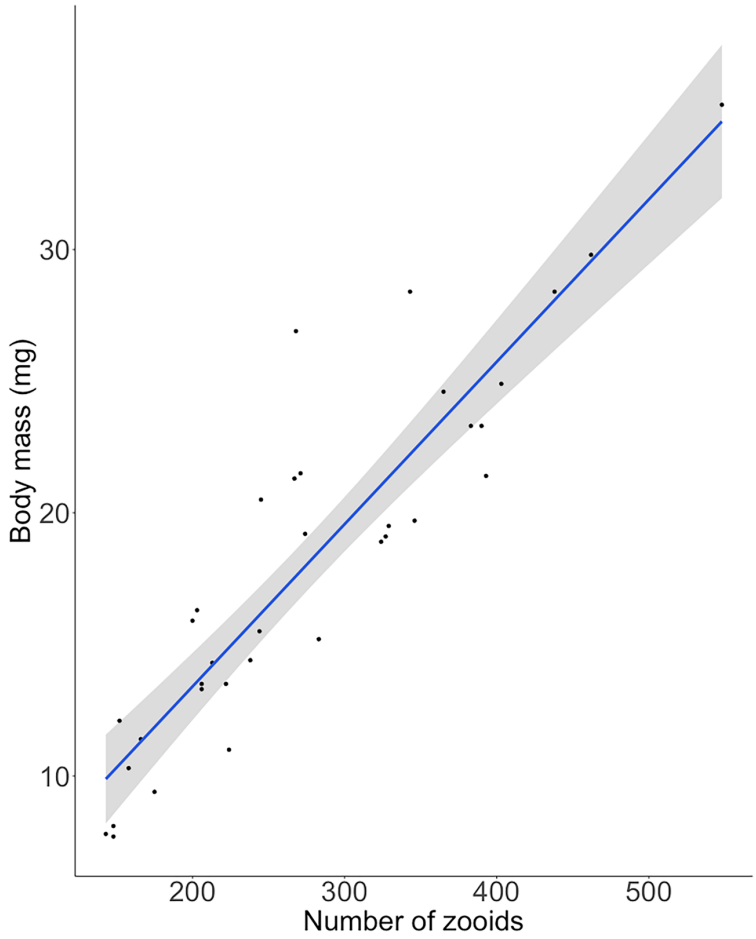

Figure S3: Relationship between the number of zooids and body mass (mg) in *Bugula neritina*. Each data point represents a single colony. The blue line indicates the linear relationship between body mass and the number of zooids. The grey area indicates the 95% confidence interval. Data derived from experiment 2b by blotting dry and weighing colonies to the nearest 0.01 g as well as counting the zooids within each colony.

Table S1: Tabulation of marine invertebrate SDA studies.

Species	Body mass (g)	T_b (°C)	Meal type	Meal size (%)	Concentration (cells ml^{-1})	Scope	Duration (h)	Treatment	Source
Bryozoa									
Bugula neritina	0.0156	19	Algae		11,500	1.43	24		present study
Cnidaria									
Astrangia danae		22	Shrimp			1.87			Szmant-Froelich and Pilson (1984)
Nemertea									
Parborlasia corrugatus	8.63	0.3	Limpet	45.5		1.7	840		Clarke and Prothero-Thomas (1997)
Mollusca									
Crepidula fornicata	0.16[a]	25	Algae		400,000	1.41		BT	Newell and Kofoed (2009)
Littorina littorea	0.08[a]	15	Algae			1.44			Shumway et al. (1993)
Littorina obtusata	0.005[a]	15	Algae			1.47			Shumway et al. (1993)
Mulinia lateralis	0.01	20	Algae		10,000	2.04	384		Shumway (1983)
Mytilus edulis	0.01[a]	15	Algae	7.3		1.5		MS	Widdows and Hawkins (1989)
Mytilus edulis	0.064[a]	15	Algae	39		1.38	10		Gaffney and Diehl (1986)
Mytilus edulis	1[a]	15	Algae		7,500	2.02			Thompson and Bayne (1972)
Mytilus edulis	1.33[a]	13.5	Algae	0.23		2.36	30		Bayne and Scullard (1977)
Nacella concinna	0.17[a]	-0.65	Algae	3.3		2.3	360		Peck and Veal (2001)
Nassarius reticulatus	0.1[a]	16	Crab			2.77			Crisp et al. (1978)
Perna canaliculus	16.9	18	Algae		400,000	1.8	96		Lurman et al. (2013)

Table S1: continued

Species	Body mass (g)	T_b (°C)	Meal type	Meal size (%)	Concentration (cells ml^{-1})	Scope	Duration (h)	Treatment	Source
Cephalopoda									
Octopus vulgaris	571	21	Crab	2.04		3	12		Wells et al. (1983)
Sepia officinalis	4.13[a]	20	Shrimp	11.2		4.32	5.7	BS, BT	Grigoriou and Richardson (2008)
Arthropoda									
Acartia tonsa	0.0000044[a]	14	Algae		27,200	2	8		Thor (2000)
Acartia tonsa	0.0000074[a]	18	Algae			5.2			Kiørboe et al. (1985)
Acartia tonsa		20	Algae			3.36			Thor et al. (2002)
Calanus euxinus	0.00107	20	Algae			1.14		BS	Svetlichny and Hubareva (2005)
Calanus finmarchicus	0.0002	14	Algae		27,200	2.8			Thor (2000)
Callinectes sapidus	195	20	Clam/fish	15		2.3	45		McGaw and Reiber (2000)
Callinectes sapidus	177.2	19	Formulated diet	1		2.6	35.3	BS	McGaw and Curtis (2013)
Callinectes sapidus	175	20	Shrimp	1		2.9		S	Curtis and McGaw (2010)
Cancer gracilis	250	11	Fish	2		2.54	55		McGaw (2006)
Cancer gracilis	169.9	11.5	Formulated diet	1.5		2.46	20.44	MS	McGaw and Curtis (2013)
Cancer irroratus	133.5	13	Formulated diet	2		2.09	21.38	BS	McGaw and Curtis (2013)
Cancer irroratus	95	15	Shrimp	2		2.7	20.3	S	Penney et al. (2016)
Cancer magister	300	12	Fish	1		1.98		S	Curtis and McGaw (2010)
Cancer magister	215	12.5	Fish	2		2.88	23.5		McGaw and Van Leeuwen (2017)
Cancer pagurus	200	11	Mussel	2		2.62			Ansell (1973)
Carcinus maenas	10	10	Squid	5.4		1.44	120		Wallace (1973)

Table S1: continued

Species	Body mass (g)	T_b (°C)	Meal type	Meal size (%)	Concentration (cells ml^{-1})	Scope	Duration (h)	Treatment	Source
Carcinus maenas	20	15	Squid	3.33		3.08	20		Robertson et al. (2002)
Carcinus maenas	36.8	15	Mussel	1.96		2.3	20		Houlihan et al. (1990)
Carcinus maenas	49	15	Mussel	4.1		1.79			Legeay and Massabuau (1999)
Carcinus maenas	53	15	Mussel	2.8		2.43	48		Mente et al. (2003)
Carcinus maenas	81.6	15	Fish	3		3.34	39.31	MT	McGaw and Penney (2014)
Carcinus maenas	80	15	Shrimp	2		2.59	17.4	S	Penney et al. (2016)
Cardisoma guanhumi	241	30	Fish	2		2.53	50		Burggren et al. (1993)
Euphausia superba	0.212	-0.5	Diatom			1.45			Ikeda and Dixon (1984)
Glyptonotus antarcticus	33	0	Krill	4.66		2.46	244.8	BT	Robertson et al. (2001a)
Gnathophausia ingens	2.8	5.5	Shrimp	2.14		1.53	7		Hiller-Adams and Childress (1983)
Goniopsis cruentata	61.4	24	Fish	3		1.75	50		Burggren et al. (1993)
Hemigrapsus nudus	38.9	14.5	Formulated diet	1.5		1.96	24.93	MS	McGaw and Curtis (2013)
Homarus americanus	3.2	20	Formulated diet			1.51		MC	Koshio et al. (1992)
Homarus americanus	454	13	Formulated diet	1.5		2.08	22.97	MS, BS	McGaw and Curtis (2013)
Jasus edwardsii	16	15	Squid	3		1.78	30		Radford et al. (2004)
Jasus edwardsii	750	13	Squid	3		1.8	42		Crear and Forteath (2000)
Leptomysis lingvura		18	Formulated diet			1.72		MT	Osma et al. (2016)
Liothyrella uva	0.29[a]	0.76	Algae			1.66	432		Peck (1996)
Litopenaeus vannamei	2.6	28	Formulated diet	2.3		1.21	4	MC	Rosas et al. (2001)

Table S1: continued

Species	Body mass (g)	T_b (°C)	Meal type	Meal size (%)	Concentration (cells ml^{-1})	Scope	Duration (h)	Treatment	Source
Maja brachydactyla		20	Squid	1		2.1		P	Thatje and Robinson (2011)
Ocypode quadrata	20.5	30	Fish	7		3.31	42		Burggren et al. (1993)
Oithona davisae	46.9	20	Algae		3,000	2.29		BS, BT	Almeda et al. (2011)
Panulirus argus	120.3	24	Fish	2		2		MT	Perera et al. (2005)
Panulirus argus	70.5	28	Formulated diet	2		1.87		BS, MT	Perera et al. (2007)
Panulirus cygnus	450	23	Squid	3		2.19	44		Crear and Forteath (2001)
Penaeus duorarum	0.031	28	Formulated diet	10		2.89		MC	Rosas et al. (1996)
Penaeus esculentus	0.27	30	Formulated diet			1.33		MC	Hewitt and Irving (1990)
Penaeus esculentus	17.7	25	Shrimp			1.39			Dall and Smith (1986)
Penaeus monodon	5.08	28	Shrimp pellet	1		1.53	5	MT, S	Du Preez et al. (1992)
Penaeus notialis	0.027	28	Formulated diet	10		3.12		MC	Rosas et al. (1996)
Penaeus schmitti	0.028	28	Formulated diet	10		2.84		MC	Rosas et al. (1996)
Penaeus setiferus	0.023	28	Formulated diet	10		2.11		MC	Rosas et al. (1996)
Penaeus setiferus	0.19	28	Formulated diet	15		2.53	6	MC	Taboada et al. (1998)
Penaeus setiferus	37.6	28	Squid	2.68		1.68	8		Rosas et al. (1995)
Procambarus clarkii	25.6	19	Formulated diet	1.5		1.78	18.5	MS	McGaw and Curtis (2013)
Pugettia producta	184.7	11.5	Formulated diet	1.5		1.66	19.91	MS	McGaw and Curtis (2013)
Saduria entomon	4.76	13	Fish	3.77		2.48	52		Robertson et al. (2001b)
Stenasellus virei	0.012	11	Meat			2.18			Hervant et al. (1997)

Table S1: continued

Species	Body mass (g)	T_b (°C)	Meal type	Meal size (%)	Concentration (cells ml^{-1})	Scope	Duration (h)	Treatment	Source
Uca pugnax	2.9	28				1.58			Vernberg (1959)
Waldeckia obesa	0.233[a]	0	Fish			4.75	7		Chapelle et al. (1994)
Annelida									
Marphysa sanguinea	4	16	Algae			1.95		BS	Yang et al. (2016)
Echinodermata									
Apostichopus japonicus (green type)	22.2	16	Formulated diet	2.17		2.18	29.6	MT	Bao et al. (2017)
Apostichopus japonicus (red type)	19.74	16	Formulated diet	2.23		2.66	31.67	MT	Bao et al. (2017)
Asteria rubens	5	15	Mussel	31		2.47	1,200		Vahl (1984)
Eucidaris tribuloides		30	Sponge			1.42			McPherson (1968)
Mellita quinquiesperforata	5[a]	25				1.37			Lane and Lawrence (1979)
Odontaster validus	5.3	0	Fish	10		1.77			Peck et al. (2008)
Pycnopodia helianthoides	2,100	11	Mussel	10		2.1	160.3	MT, MS	McGaw and Twitchit (2012)
Sterechinus neumayeri	0.79[a]	-0.3	Fish	4		1.24		BT, pH	Morley et al. (2016)
Strongylocentrotus droebachiensis	44	14	Algae			2.11			Lilly (1979)
Strongylocentrotus droebachiensis	35.5	10	Kelp			1.83		MT	Orr et al. (2014)

Table S1: continued

Species	Body mass (g)	T_b (°C)	Meal type	Meal size (%)	Concentration (cells ml^{-1})	Scope	Duration (h)	Treatment	Source
Rotifera									
Brachionus plicatilis		25	Formulated diet			3.53		MC	Osma et al. (2016)
Urochordata									
Ciona intestinalis	0.1	15	Algae		9,400	6.55	53		Sigsgaard et al. (2003)

Meal size (%) is reported as a percentage of body mass.

T_b body temperature; studies with experimental treatments are noted as *BS* body size, *BT* experimental temperature, *MC* meal composition, *MS* meal size, *MT* meal type, *S* salinity, *P* pressure

Studies, for which concentration and/or scope were not reported, presented values were calculated from published information.

[a] Body mass reported as dry mass

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Table S2: Non-significant interactions excluded from the final model examining the effects of mass and treatment (size-manipulated vs. intact) on the Routine MR of *B. neritina* colonies during starvation.

	df	**F**	***P***
Within subjects			
$\log_{10}$(Mass) × time	3	1.391	0.247
$\log_{10}$(Mass) × treatment × time	3	0.647	0.586

Table S3: Non-significant interactions excluded from the final model examining the effects of mass, treatment (size-manipulated vs. intact) and feeding (starved vs. fed) on the Routine MR of *B. neritina* colonies.

	df	**F**	***P***
Between subjects			
$\log_{10}$(Mass) × treatment	1	1.031	0.319
$\log_{10}$(Mass) × feeding	1	2.303	0.141
Treatment × feeding	1	0.295	0.591
$\log_{10}$(Mass) × treatment × feeding	1	0.384	0.541
Within subjects			
$\log_{10}$(Mass) × treatment × time	6	1.03	0.408
$\log_{10}$(Mass) × time × feeding	6	0.815	0.559
Treatment × time × feeding	6	0.767	0.597
$\log_{10}$(Mass) × treatment × time × feeding	6	0.754	0.607

Table S4: Non-significant interactions removed from the final model examining the effects of mass and treatment (size-manipulated vs. intact) on the Routine MR of *B. neritina* colonies during SDA.

	df	**F**	***P***
Between subjects			
$\log_{10}$(Mass) × treatment	1	0.012	0.913
Within subjects			
$\log_{10}$(Mass) × time	3	1.515	0.213
$\log_{10}$(Mass) × treatment × time	3	0.253	0.859

Chapter 3

Plastic but not adaptive: environmentally-driven differences in metabolic rate despite consistent selection

Abstract

Metabolic plasticity in response to changing environmental conditions is widespread across taxa and often observed in the field. Therefore, it is reasonable to expect that such plasticity should be adaptive, but only few studies have determined the adaptive significance of metabolic plasticity by formally estimating selection on metabolic rate under different environmental conditions. We used a model marine colonial invertebrate, *Bugula neritina* to examine selection on metabolic rate in a harsh and a benign environment in the field and tested whether these environments induced the expression of different metabolic phenotypes. We conducted two experimental runs and found evidence for positive correlational selection on the combination of metabolic rate and colony size in both environments in one run, whereas we could not detect any selection on metabolic rate in the second run. Even though selection did not differ between environments, we found evidence for plasticity in metabolic rate – colonies expressed different metabolic phenotypes depending on the environment they experienced. In other words, we found evidence for phenotypic plasticity in metabolic rate, but this plasticity was not adaptive.

Introduction

Metabolic rate determines the rate at which organisms transform resources from the environment, use energy and live (Auer et al. 2018, Brown et al. 2004, Hulbert and Else 2000, Pettersen et al. 2018, but see Glazier 2015). Metabolic rate varies at all levels – among species, populations and conspecifics, even after accounting for differences in body mass or temperature (Burton et al. 2011, Konarzewski and Książek 2013, White and Kearney 2013). Over the last few decades, metabolic theory has sought to explain the drivers of variation in metabolic rate in natural populations (Burton et al. 2011, Pettersen et al. 2018).

Metabolic rate, like many traits, is plastic; it changes when conditions change (Norin and Metcalfe 2019). Temperature is the most obvious and perhaps strongest driver of metabolic plasticity but there are many others (Clarke 2017). For example, metabolic rate has been shown to vary with resource availability in a range of taxa – individuals increase their metabolic rate when resources are abundant but decrease them when resources are limited (Auer et al. 2015, Guppy and Withers 1999, Mueller and Diamond 2001, Naya et al. 2009, O'Connor 2000, Schimpf et al. 2012). In the field, such reduced metabolic rates are often found in high-density populations where competition imposes a key constraint on the availability of resources (Antonovics and Levin 1980, Ghedini et al. 2017, Violle et al. 2010). Although recent studies have shown that organisms in a variety of taxa express different metabolic rates in response to different environmental conditions, the degree to which this covariation is adaptive remains largely unclear.

Phenotypic plasticity can be regarded as adaptive if organisms respond to environmental change by expressing the phenotype that is in the same direction as the optimal value favoured by selection in the new environment (DeWitt and Scheiner 2004, Ghalambor et al. 2007, Pigliucci 2001). In addition, selection needs to favour different phenotypes in the different environments, such that no metabolic phenotype is superior across

all environments (DeWitt and Scheiner 2004, Ghalambor et al. 2007, Pigliucci 2001). For example, if individuals reduce their metabolic rates in response to resource limitation and selection favours lower metabolic rates in that environment but a lower metabolic rate is not advantageous in a resource rich environment, then it would be reasonable to conclude that this plasticity is adaptive. Thus, in order to determine whether a change in metabolic rate across environments is adaptive, one needs to first estimate how selection on metabolic rate varies among environments, then estimate how metabolic rate changes across environments.

There is some evidence for adaptive metabolic plasticity. Auer et al. (2015) and Zeng et al. (2017) showed that, in brown trout and juvenile qingbo, respectively, individuals that had increased or decreased metabolic rates in response to elevated or restricted resource levels grew the most. Similarly, in response to food scarcity, individuals with the greatest reduction in metabolic rate lost the least amount of fat in a simulated overwintering scenario (Auer et al. 2016). Handelsman et al. (2013) found that, in Trinidadian guppies, individuals reduced their metabolic rates in response to predator cues, and this plasticity was in the same direction as evolution. Thus, there seems to be evidence for adaptive metabolic plasticity under laboratory conditions at least. In the field, however, few studies have formally estimated selection on metabolic rate under different environmental conditions (Pettersen et al. 2018). Furthermore, most studies necessarily rely on fitness proxies such as survival or growth rather than the reproductive output of an individual, i.e. reproductive fitness. Estimates of fitness that include reproductive outputs are not always accessible but are more likely to fully characterise selection (Pettersen et al. 2018).

Recent evidence suggests that metabolic rate is a target of selection in the wild (reviewed in Pettersen et al. 2018). Across taxa, metabolic rate has been shown to be heritable to some extent and is, therefore, likely to evolve under selection (Auer et al. 2018, Pettersen et al. 2018, White et al. 2019). Phenotypic selection is the covariance between a

trait and relative fitness, where fitness is determined as an individual's contribution of offspring to the next generation (Falconer and Mackay 1996). Selection can be estimated by using a linear regression framework, as elegantly shown by the fundamental work of Robertson (1966), Price (1970), and Lande and Arnold (1983). Here, the slope of the relationship between relative fitness and a trait, weighted by the phenotype distribution, represents standardized estimates of selection (Lande and Arnold 1983). Nevertheless, selection likely acts on combinations of traits, rather than traits in isolation (Blows and McGuigan 2015, Lande and Arnold 1983). Most traits, such as metabolic rate, are genetically correlated with other traits that affect fitness (Auer et al. 2017, Mathot et al. 2019). For example, metabolic rate is correlated with body mass (White et al. 2019), growth rate (Sadowska et al. 2009), or exploratory behaviour (Biro and Stamps 2010, Careau et al. 2011) in a range of species. If two traits are correlated, estimates of selection on one trait will likely result in misleading conclusions since apparent selection on one trait may be due to selection on another unmeasured, correlated trait (i.e. indirect selection; Lande and Arnold 1983). In order to overcome these limitations, the use of a multi-trait selection framework is necessary.

Here, I examined (i) phenotypic selection on metabolic rate in a benign and a harsh environment, and (ii) metabolic plasticity in response to a shift from the benign to the harsh environment in the colonial bryozoan *Bugula neritina* in the field. Note that in order to determine the adaptive value of phenotypic plasticity, phenotypic selection needs to be estimated in both environments. Estimating selection in one environment only or on the magnitude of phenotypic plasticity (i.e. the reaction norm) is inadequate since both environments may favour the same phenotype, in which case less plasticity may be the optimal strategy, rendering plasticity non-adaptive (DeWitt and Scheiner 2004). To our knowledge, few studies of metabolic plasticity have completed these essential steps for determining the adaptive consequences of any observed plasticity. I took advantage of the

sessile nature of *B. neritina*, which is commonly found as part of the fouling community on piers throughout the world. Depending on whether colonies grow on the side or the underside of the pier, they experience either a harsh environment (when growing on vertical surfaces), in which individuals are exposed to higher sedimentation rates and higher levels of UV radiation, such that they grow and reproduce less, or a benign environment (when growing on horizontal surfaces) (Hart and Marshall 2013). Colonies of *B. neritina* produce free-swimming, non-feeding larvae that are immediately competent to settle following release, and most settle within hours under field conditions (Burgess and Marshall 2011). Although larvae may settle only centimetres apart from each other, they can end up in very distinct environments, in which selection regimes may differ considerably (Marshall and Monro 2013, Pettersen et al. 2020). Furthermore, since *B. neritina* colonies are sessile, I highlight the tractability of this system to follow growth, survival, and lifetime reproductive outputs in the field. I formally estimated parameters related to selection, including the opportunity for selection (I), and linear (β) and non-linear (γ) selection gradients in the different environments across two experimental runs. By estimating phenotypic selection parameters combined with estimates of phenotypic plasticity, I was able to investigate whether metabolic plasticity is adaptive in *B. neritina* in the field.

Materials and methods

Study species, site and field deployment

The colonial bryozoan *Bugula neritina* Linnaeus, 1758, is common to sessile marine communities worldwide. Colonies grow by asexual budding of new zooids (individual subunits) at the distal ends such that, within a colony, individual zooids are genetically identical. After approximately every four pairs of zooids, colonies form regular bifurcations to produce symmetrical branching (Keough and Chernoff 1987, Keough 1989). Once

colonies reach sexual maturity, they form clearly visible, calcified structures called ovicells (Woollacott and Zimmer 1975). Each ovicell broods a single larva, which is released into the plankton once embryogenesis is complete. Upon release, the non-feeding larvae are immediately competent to settle and grow into a new, individual colony.

I collected sexually mature *B. neritina* colonies from the Royal Melbourne Yacht Squadron in Port Phillip Bay, Victoria, Australia (-37.865, 144.966) in March and April 2018. To obtain individuals for my experiments, I spawned colonies according to standard procedures (Schuster et al. 2019). Briefly, I kept colonies in the laboratory in field-collected seawater in aerated tanks in the dark. After 48h, I spawned colonies by exposing them to bright light and settled single larvae in a drop of seawater on roughened A4 acetate sheets to induce settlement (~150 settlers per acetate sheet). After three hours, I rinsed unsettled larvae from the acetate sheets and kept settlers in tanks with unfiltered seawater. The next day, I attached two A4 acetate sheets bearing settlers to 20 PVC backing panels (57 × 57 × 0.6 cm) across two experimental runs (experimental run 1: eight panels; experimental run 2: 12 panels). I initiated the two runs four weeks apart to provide a larger sample size and to explore whether any observed effects were consistent over time. In both runs, I suspended the panels 1 m below the water surface with settlers facing down at the Royal Brighton Yacht Club (-37.909, 144.986).

Mass-independent metabolic rate

I estimated selection on mass-independent metabolic rate. Note that this is not synonymous with mass-specific metabolic rate (i.e. metabolic rate divided by body mass). I calculated mass-independent metabolic rate (MI-MR) by regressing metabolic rate on colony mass (nonlinear regression of the form $MR = a * M^b$, where MR is metabolic rate, M is colony

mass, a is the intercept, and b is the scaling exponent) within each panel and extracting the residuals (hereafter metabolic rate early, MI-MR_E).

To conduct metabolic rate measurements, I returned acetate sheets bearing settlers to the laboratory after colonies within each experimental run had been in the field for three weeks. I kept colonies in aerated tanks with field-collected seawater at 19°C overnight. Prior to metabolic rate measurements, I removed any epibionts and debris from the colonies. I then separated individual colonies from the A4 sheets by cutting around the base of the colonies such that each colony was attached to a small square of acetate sheet. I measured metabolic rate using 5 ml (run 1) or 750 µl (run 2) glass vials (Loligo Systems, Denmark) at 19 °C as described in Schuster et al. (2019).

To determine colony size of three weeks old colonies (hereafter original colony size), I counted the number of zooids in each colony – in my experience this is a more reliable estimate of mass for field-collected colonies that are growing on acetate, but the number of zooids and colony mass are strongly correlated (Schuster et al. 2019). Colonies used for metabolic rate measurements ranged from 112 to 372 zooids in size in run 1 (four to six bifurcations) and from 20 to 80 zooids in run 2 (two to four bifurcations). Note that these differences in colony size between runs arose due to higher sea surface temperatures combined with higher growth rates in March (run 1) compared to April (run 2). In both runs, colonies were three weeks old when I conducted measurements of colony size and metabolic rate.

Following metabolic rate measurements, I glued each colony onto a 25 cm^2 single acetate sheet, which I then assigned to a PVC plate (55 × 55 × 3 mm) with a unique ID number. I attached the plates, each bearing one single colony with known MI-MR, onto their initially assigned panels (total number of panels, N = 20), with up to 16 plates per panel (run 1: N = 121; run 2: N = 175; total number of colonies deployed, N = 296). I redeployed the

panels back into the field, but placed half of the panels (N = 10) into a vertical position with colonies facing sideways. Here, vertically deployed panels (in contrast to horizontally deployed panels) represent a harsh environment for *B. neritina*, with colonies being exposed to higher sedimentation rates and higher levels of UV radiation, such that they grow and reproduce less (Hart and Marshall 2013). Additionally, to avoid confounding depth with orientation, I attached colonies on vertically suspended panels at a similar depth to horizontally suspended panels (see Fig. S1 for details on deployment). It is noteworthy that I assigned colonies haphazardly to each environment; consequently, there were no differences in colony size or MI-MR between environments in run 1 (mean ± SE; benign environment: zooids: 231.13 ± 7.36, MI-MR: -0.01 ± 0.55; harsh environment: zooids: 235.67 ± 7.16, MI-MR: 0.01 ± 0.53; t-test: zooids: t_{119} = -0.44, P = 0.66, MI-MR: t_{119} = -0.01, P = 0.99) or run 2 (mean ± SE; benign environment: zooids: 42.25 ± 1.38, MI-MR: 0.01 ± 0.24; harsh environment: zooids: 43.26 ± 1.66, MI-MR: 0.02 ± 0.26; t-test: zooids: t_{173} = -0.46, P = 64, MI-MR: t_{173} = -0.04, P = 0.97).

Performance measures

I followed survival, fertility (colonies that survived to reproduce), and the reproductive output of each colony in the field every two weeks over the entire life history, until all colonies had died (March through to October 2018). Colonies were considered alive if they were still attached to the plate and >10% of the colony contained feeding zooids. I measured the reproductive output of each colony as the cumulative number of ovicells throughout the duration of the experiment. In addition, I measured three fitness-related traits: growth (the number of bifurcations as an indication of colony size; Keough and Chernoff 1987), age at onset of reproduction, and longevity (number of weeks >10% alive).

To avoid any environmental effects associated with a colony's position within a panel on metabolic rates or performance, I moved each plate to a different position within the assigned panel every two weeks (Mitchell-Olds and Shaw 1987, Rausher 1992). I randomised the position of each colony within their assigned panel only, I did not move colonies across panels. I accounted for any panel effects in statistical analyses by including panel as a random effect, nested within environment and run, in all models.

Estimates of selection on metabolic rate early and original colony size

a) Testing for differences in reproductive outputs among environments and experimental runs

To determine whether colony reproductive outputs differed among environments and experimental runs, I used a linear mixed effects model to test for the effects of run, environment, and their interaction on the reproductive output of *B. neritina* colonies.

b) Estimating the opportunity for selection in the different environments

I estimated the opportunity for selection (I) within each environment and run. The opportunity for selection is a measure for the amount of absolute variation in fitness within a population and is calculated as $I = \sigma_W^2/\overline{W}^2$, where σ_W^2 is the variance in absolute fitness and $\overline{W}$ is the mean absolute fitness (Crow 1958). I calculated bootstrap confidence intervals using the R package *boot* version 1.3-24 (Canty and Ripley 2019, Davison and Hinkley 1997).

c) Testing for differences in colony fertility

Colony fertility (i.e. the number of colonies that survived to reproduce) was 100% in experimental run 1, whereas only three out of 175 colonies died before they reproduced in run 2. Therefore, I did not statistically test for effects of MI-MR_E, original colony size, or environment on colony fertility due to lack of variance in the response variable.

d) Characterizing fecundity selection within and between environments

To characterize selection within and differences in selection between environments, I used estimates of metabolic rates derived from colonies grown in a common environment (i.e. MI-MR_E) rather than metabolic rates of colonies exposed to the benign or harsh environment. I did so, because metabolic rates measured in different environments represent different traits (Falconer and Mackay 1996), making inferences about differences in phenotypic selection on metabolic rate between environments invalid. Furthermore, given that original colony size differed between experimental runs, I analysed each run separately due to non-overlapping covariance ranges. To estimate selection on MI-MR_E and original colony size, I used multiple regression to estimate the relationship between relative fitness (an individual's lifetime reproductive output divided by the average lifetime reproductive output of all colonies within a given panel) and my standardized traits of interest (Lande and Arnold 1983). I standardized fitness within each panel because colony reproductive outputs differed across panels (run 1: $\chi^2 = 10.56$, df = 1, $P = 0.001$; run 2: $\chi^2 = 19.77$, df = 1, $P = 8.75 \times 10^{-6}$). In run 2, six colonies did not reproduce, three of which did not survive to reproduce. I included these six colonies as "0" fitness in selection analyses since the overall qualitative outcome did not change if I excluded these colonies from analyses. MI-MR_E and original colony size were not significantly correlated (run 1: $r = 0.002$, $P = 0.98$; run 2: $r = -0.02$, $P = 0.75$).

In all selection analyses, I included panel as a random effect, nested within environment (Mitchell-Olds and Shaw 1987). To begin with, I conducted an overall, formal test of whether selection differed between environments (benign vs. harsh). I compared models in which selection coefficients differed between environments to models in which selection was assumed to be constant between environments. As outlined by Chenoweth and Blows (2004), I used a sequence of model comparisons to (1) establish a baseline model to account for environmental effects on fitness; (2) test whether linear selection gradients

systematically differed between environments; and (3) test whether nonlinear selection gradients systematically differed between environments. I then estimated standardized linear (β) and nonlinear (γ) selection gradients using relative fitness and standardized traits (Lande and Arnold 1983, Phillips and Arnold 1989). To produce corresponding estimates of terms in the γ matrix, I doubled estimated coefficients of the quadratic terms (Stinchcombe et al. 2008).

Environment-dependent covariance between MI-MR_E and life-history traits

Metabolic rate is linked to a range of key life-history traits, which together mediate an individual's pace of life (Auer et al. 2018, but see Glazier 2015). Hence, to understand how selection on metabolic rate might be mediated through its effect on the pace of life, I measured three key life-history traits.

a) Growth

I tested for the effects of run, environment, and MI-MR_E on growth (number of bifurcations over time) during the first 25 weeks using a repeated measures analysis of covariance (RM ANCOVA). I included run, environment, and time (measurement points) as categorical fixed effects, and MI-MR_E as the covariate of interest.

b) Age at onset of reproduction

Onset of reproduction differed across experimental runs. In run 1, colonies first reproduced after three weeks, whereas colonies in run 2 developed ovicells after nine weeks in the field. Therefore, I considered colonies that had developed ovicells at three weeks (run 1) or at nine weeks (run 2) to have an early onset of reproduction and assigned them a "1", while colonies noted to develop ovicells later on were denoted "0". I then fit a logistic regression to the data, including run, environment, MI-MR_E, and their interaction as fixed effects.

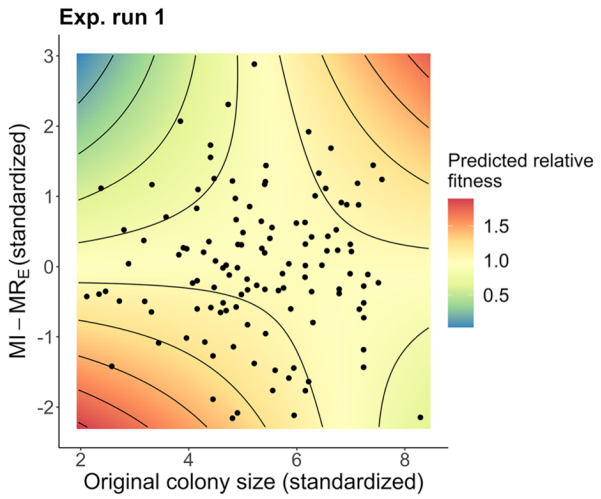

Figure 1: Predicted relative fitness (total lifetime reproductive output) plotted against mass-independent metabolic rate early (MI-MR$_E$) and original colony size for *Bugula neritina* colonies in experimental run 1 (N = 121). Black dots represent the underlying data points. Warmer colours indicate higher relative fitness.

c) Longevity

To determine colony longevity, I assigned "0" or "1" to colonies that survived less than or more than 20 weeks in the field, respectively (c.f. Pettersen et al. 2016, Pettersen et al. 2020). I analysed the longevity data using a logistic regression as described above.

Testing for phenotypic plasticity in metabolic rate

I was interested in how the environment (benign vs. harsh) affected the colonies' metabolic rates. Hence, I returned all colonies to the laboratory for a second metabolic rate measurement (hereafter mass-independent metabolic rate late, MI-MR$_L$) after they had been in the respective environments for two weeks (colonies were five weeks of age). I determined MI-MR$_L$ as described above and redeployed colonies at the Royal Brighton Yacht Club

Table 1: Linear mixed effects model for the relationship between total reproductive output and mass-independent metabolic rate early ($MI\text{-}MR_E$) and original colony size. I included panel as a random effect, nested within environment, in all analyses. All *df* = 1.

	χ^2	*P*
Exp. run 1		
Environment	2.61	0.11
$MI\text{-}MR_E$	0.69	0.41
Original colony size	2.66	0.1
Environment × $MI\text{-}MR_E$	0.03	0.85
Environment × original colony size	3.07	0.08
$MI\text{-}MR_E^2$	0.02	0.88
Original colony size^2	1.48	0.22
Environment × $MI\text{-}MR_E^2$	2.09	0.15
Environment × original colony size^2	0.31	0.58
$MI\text{-}MR_E$ × original colony size	5.47	**0.02**
Environment × $MI\text{-}MR_E$ × original colony size	0.83	0.36
Exp. run 2		
Environment	16.22	$\mathbf{5.6 \times 10^{-5}}$
$MI\text{-}MR_E$	0.22	0.64
Original colony size	20.29	$\mathbf{6.6 \times 10^{-6}}$
Environment × $MI\text{-}MR_E$	1.77	0.18
Environment × original colony size	0.01	0.92
$MI\text{-}MR_E^2$	1.27	0.26
Original colony size^2	0.92	0.34
Environment × $MI\text{-}MR_E^2$	0.05	0.83
Environment × original colony size^2	0.28	0.59

MI-MR_E × original colony size	0.21	0.64
Environment × MI-MR_E × original colony size	1.32	0.25

afterwards. Colonies that were initially placed into a harsh environment were again attached to vertically suspended panels whereas colonies initially placed into a benign environment were reattached to horizontally suspended panels. I then used a linear mixed effects model to test for the effects of MI-MR_E, environment, and their interaction on MI-MR_L. I included MI-MR_E and environment as fixed effects.

I conducted all analyses in R version 3.6.2 (R Core Team 2017) using the packages *nlme* (Pinheiro et al. 2017) and *lme4* (Bates et al. 2007). I reduced each model by removing non-significant interactions ($P > 0.05$) if their inclusion did not improve the model fit (Quinn and Keough 2002).

Results

Variation in reproductive outputs and the opportunity for selection in each environment

The effect of environment (benign vs. harsh) on colony reproductive outputs differed between experimental runs ($F_{1,16} = 5.69$, $P = 0.03$). In run 1, *per capita* reproductive outputs were on average 26.62% lower in the harsh environment, whereas colonies had on average 50.08% fewer ovicells in the harsh environment in run 2 (Fig. S2). Furthermore, the opportunity for selection (I) was higher in the harsh environment in both runs (Table S1). $I(harsh)$ ranged between 0.43 ($CI_{95\%}$: 0.26, 0.62; run 1) and 0.59 ($CI_{95\%}$: 0.41, 0.77; run 2), while $I(benign)$ ranged between 0.33 ($CI_{95\%}$: 0.2, 0.47; run 1) and 0.37 ($CI_{95\%}$: 0.26, 0.48; run 2).

Table 2: Selection coefficients (± SE) for mass-independent metabolic rate early (MI-MR_E) and original colony size with total reproductive output (cumulative number of ovicells) for *Bugula neritina* colonies. β and γ represent linear and nonlinear selection gradients, respectively (* $P < 0.05$; *** $P < 0.0001$).

		γ' (± SE)	
Exp. run 1	β' **(± SE)**	*Original colony size*	*MI-MR_E*
Original colony size	0.0006 (0.037)	-0.008 (0.047)	**0.087** (0.04) *
MI-MR_E	-0.067 (0.05)		0.013 (0.073)
Exp. run 2			
Original colony size	**0.199** (0.046) ***	-0.043 (0.089)	-0.028 (0.046)
MI-MR_E	-0.008 (0.049)		-0.055 (0.078)

Selection on MI-MR_E and original colony size

In both experimental runs, selection on MI-MR_E and original colony size did not differ between environments (no significant environment × $MIMR_E$ or environment × original colony size interactions; Table 1). In run 1, I could not detect linear selection acting on either MI-MR_E or original colony size, but I found support for significant non-linear selection (Tables 1 and 2). When exploring the different forms of non-linear selection, I could not detect any quadratic selection, but I detected correlational selection acting on the combination of MI-MR_E and original colony size. Here, positive correlational selection indicated that smaller colonies with lower mass-independent metabolic rates, and larger colonies with higher mass-independent metabolic rates had the highest fitness (Fig. 1). In run 2, I found support for positive linear selection acting on original colony size, with larger colonies having the highest fitness (Tables 1 and 2). I could not detect any linear selection acting on

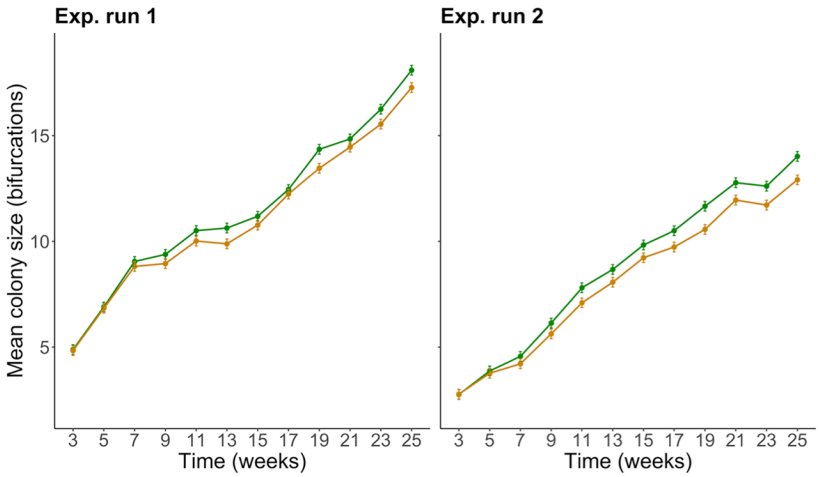

Figure 2: Mean colony size (as mean number of bifurcations) in the benign (green lines) and the harsh (orange lines) environment plotted against time (in weeks). Error bars show standard errors.

MI-MR_E and I found no support for non-linear selection acting on either MI-MR_E or original colony size (Table 1).

Environment-dependent covariance between MI-MR_E and life-history traits

a) Growth

During the first 25 weeks, I could not detect an effect of MI-MR_E on colony size. The harsh environment, however, negatively affected colony size in both runs, and the effect strengthened over time (Fig. 2; Table S2). At 25 weeks, colonies in the harsh environment were on average 7.15% smaller than colonies in the benign environment.

b) Age at onset of reproduction

The effect of environment on age at onset of reproduction depended on experimental run (χ^2 = 3.95, df = 1, P = 0.04). In run 2, colonies in the benign environment developed ovicells earlier, on average after 10.08 (± 0.24 SE) weeks in the field, whereas colonies in the harsh

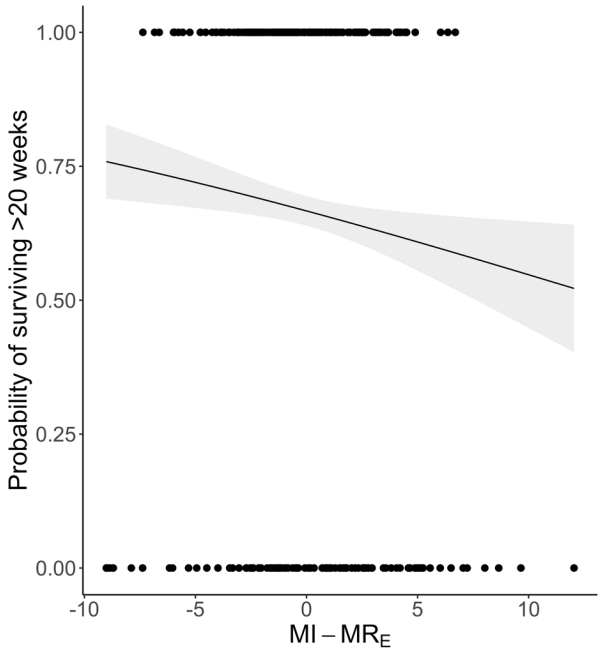

Figure 3: Logistic regression for predicted longevity (probability of surviving >20 weeks) plotted against mass-independent metabolic rate early (MI-MR_E). Data points represent predicted MI-MR_E for each colony.

environment developed ovicells on average after 11.6 (± 0.34 SE) weeks. In run 1, age at onset of reproduction did not differ between environments, with colonies on average reproducing after 4.72 (± 0.08 SE) weeks. MI-MR_E did not affect age at onset of reproduction in either run (χ^2 = 0.55, df = 1, P = 0.46).

c) Longevity

Low metabolic rate colonies lived longer than higher metabolic rate colonies in both runs (χ^2 = 4.42, df = 1, P = 0.04; Fig. 3), and the effect did not differ between environments (χ^2 = 0.38, df = 1, P = 0.54).

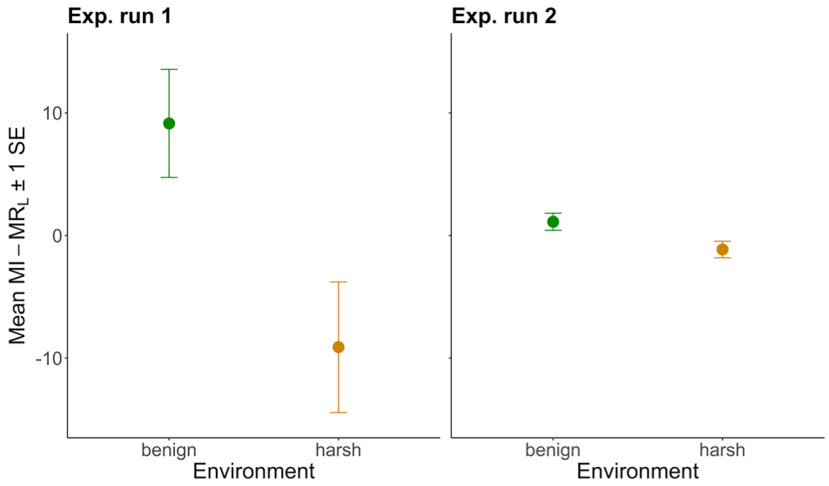

Figure 4: Mean mass-independent metabolic rate late (MI-MR$_L$ ± 1 SE; at 5 weeks) in the benign (green) and harsh (orange) environment, respectively, in each experimental run.

Metabolic plasticity: the effects of environment and MI-MR$_E$ on mass-independent metabolic rate late (MI-MR$_L$)

The degree of metabolic plasticity differed between experimental runs ($F_{1,16} = 6.1$, $P = 0.03$). In run 1, colonies in the harsh environment had on average 95.31% lower mass-independent metabolic rates at five weeks than colonies in the benign environment. In run 2, the difference was more subtle, with colonies in the harsh environment having on average 72.38% lower mass-independent metabolic rates (Fig. 4). Furthermore, MI-MR$_E$ significantly affected MI-MR$_L$ ($F_{1,275} = 8.36$, $P = 0.004$), with initially higher metabolic rate individuals also having higher metabolic rates later on, and vice versa. The effect of MI-MRE did not differ between environments ($F_{1,273} = 0.16$, $P = 0.69$) or experimental runs ($F_{1,274} = 3.24$, $P = 0.07$). I also could not detect a significant MI-MR$_E$ × environment × run interaction ($F_{1,272} = 0.01$, $P = 0.93$).

Discussion

I found no differences in selection between the harsh and the benign environment, but colonies expressed different metabolic phenotypes in those environments. Together, these results imply that, although environmental variation can induce changes in metabolic rate, these changes are not necessarily adaptive. My results highlight the importance of using a formal framework as recommended by DeWitt and Scheiner (2004) for evaluating whether phenotypic plasticity is indeed adaptive or not. Given the strong and consistent metabolic response to the environmental manipulation that I observed, it would have been tempting to infer that such a response increases fitness, but my comprehensive mapping of metabolic phenotype to fitness across environments contradicts this intuition. My results are not a product of a lack of statistical power – earlier studies in my system find differences in selection on metabolic rate with lower levels of replication (Pettersen et al. 2020). While such results are less intuitively appealing than findings of adaptive plasticity, it is important not to misrepresent the ubiquity of adaptive plasticity in metabolic rate by deemphasising studies that find no evidence for it.

Variation in environmental conditions such as resource availability is ubiquitous in nature. Given the strong co-dependence of metabolic rate and feeding (Guppy and Withers 1999, O'Connor et al. 2000, Mueller and Diamond 2001, Naya et al. 2009, Schimpf et al. 2012, Auer et al. 2015), one might expect that adjusting the metabolic phenotype to prevailing conditions would confer fitness advantages. I found that colonies in the harsh environment had much reduced metabolic rates, but a lower metabolic rate was not advantageous in that environment. Furthermore, I could not detect any differences in selection between environments overall. Yet, it is unlikely that I was unable to detect any environmental variation in selection due to low statistical power, since I found some evidence for selection acting on metabolic rate and (or) colony size in both experimental runs.

Phenotypic plasticity is often assumed to have evolved as an adaptation to environmental heterogeneity, but many plastic phenotypes are the consequences of a 'passive' response to environmental stress (van Kleunen and Fischer 2005). Such passive responses may evolve due to genetic correlations with other traits that are under selection or due to genetic drift (van Kleunen and Fischer 2005), are likely non-adaptive, and can even be maladaptive (Schmalhausen 1949, Smith-Gill 1983, Thompson 1991, Schlichting and Pigliucci 1995). Hence, metabolic plasticity may merely represent a passive response due to correlations with other traits, but further studies estimating metabolic plasticity combined with formal measures of selection on metabolic rate in different environments are needed to uncover whether metabolic plasticity may be adaptive in other species.

Metabolic rate and body size are strongly correlated (White et al. 2019). Yet, I show that, when accounting for body size effects on metabolic rate, mass-independent metabolic rate and body size can interact to affect individual fitness. Specifically, I found that both a low and a high metabolic rate can be advantageous within a population, but it depends on colony size. In aquatic systems (including my own), the physical structure of sessile organisms can disrupt boundary currents and increase resource entrainment, particularly at larger body sizes (Okamura 1984). Larger individuals are also more likely to overcome boundary layers and access different resource pools, thereby increasing their overall access to resources (Okamura 1984). I found that larger colonies only had relatively higher fitness if they also had relatively higher metabolic rates. Individuals with higher metabolic rates are thought to have faster physiologies, which may allow them to forage more voraciously or effectively, such that they can extract more resources from the environment (McNab 1980, Chappell et al. 2007, Biro and Stamps 2010). A higher resource intake, in turn, may allow for a higher sustained energy throughput and result in increased fitness (Burton et al. 2011). Conversely, smaller colonies that were more limited in their access to resources had a

relatively higher fitness if their metabolic demands were relatively low. Taken together, these findings indicate that the benefits of a metabolic phenotype depend on other trait values such as body size, suggesting that metabolic rate is unlikely to evolve independently of other traits (White et al. 2019, Kozłowski et al. 2020).

Recent studies have shown that metabolic rate covaries with fitness in a range of species in the field (reviewed in Pettersen et al. 2018) – so why did I not detect strong linear or quadratic selection on metabolic rate overall? In the wild, phenotypic selection is not a constant process, but it can fluctuate on both a temporal and spatial scale (Bell 2010). In line with these findings, I found that selection differed across experimental runs. In the long run, however, only spatial variation in selection is predicted to lead to trait differentiation among populations, whereas temporal variation should slow the evolution of varying phenotypes (Levins 1968, Bell 1997). Moreover, metabolic rate is not a single trait – there are various types of metabolic rates that selection can act on, such as resting metabolic rate or maximum metabolic rate (Suarez 2012). Thus, an organism has no single metabolic rate and selection likely perceives them (and their combinations) differently (Pettersen et al. 2016).

Other studies have found strong selection on metabolic rate in my species (Pettersen et al. 2016, Pettersen et al. 2020), whereas I did not. These differences may be due to the measurements of metabolic phenotypes at different life-history stages, which differ in the potential for the environment to affect metabolic rates (e.g., Withers et al. 2006, White et al. 2007, Jetz et al. 2008, Alton et al. 2012, Naya et al. 2018). For example, Pettersen et al. (2016) and Pettersen et al. (2020) measured metabolic rates during the larval stage, whereas I determined metabolic rates of three weeks old colonies that had been in the field prior to measurements. Although metabolic rate is generally repeatable, especially over short timescales (White et al. 2013), estimates of repeatability are usually lower under field conditions due to greater environmental variability (Auer et al. 2016). Thus, the metabolic

rates I measured were likely a product of small-scale environmental differences rather than the underlying metabolic phenotype of the organisms. My findings, therefore, pertain to the metabolic phenotypes of three weeks old individuals as my trait of interest. Nevertheless, measuring metabolic rate early in the life history, before environmental effects have a chance to influence it, may provide a better measure of the intrinsic metabolic phenotype.

How may environmental heterogeneity alter the process and outcome of selection on metabolic rate? I found that colonies in the harsh environment performed more poorly, but the overall variation in relative fitness was much higher than in the benign environment. Accordingly, the opportunity for selection was greater in the harsh environment, which can be indicative of an increased selection intensity combined with a greater potential for evolutionary change (Crow 1958, Arnold and Wade 1984, Jones 2009). Nevertheless, I could not detect any differences in the intensity or form of selection acting on metabolic rate in the different environments. Therefore, my findings suggest that although environmental heterogeneity has the capacity to alter variation in fitness, it may not affect the distribution of metabolic phenotypes in my system.

Metabolic plasticity in response to environmental perturbation such as changes in temperature or resource availability has been observed in a range of species (Norin and Metcalfe 2019). Nevertheless, to my knowledge, no studies have formally tested the adaptive significance of such metabolic plasticity in the field. When traits differ dramatically among environments, it is tempting to infer that such differences are driven by adaptive plasticity. Yet, I find that even though colonies expressed very different metabolic phenotypes in the benign and the harsh environment, there is no evidence that this differential expression is adaptive. Instead, it seems that environments can induce changes in metabolic rates in nominally non-adaptive ways. Nevertheless, additional studies investigating environmental variation in selection on metabolic rate combined with measures of metabolic plasticity are

needed in order to understand the drivers and consequences of metabolic plasticity in the field.

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Supplementary material

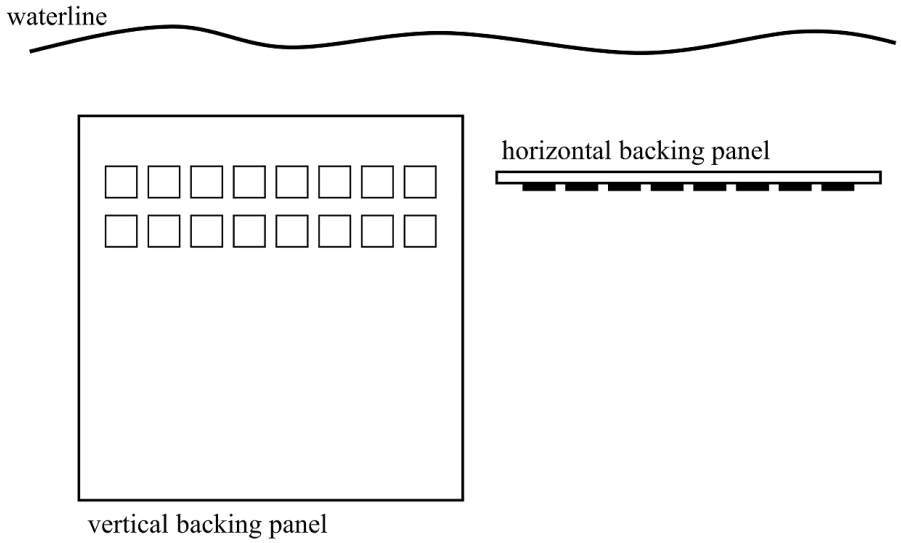

Figure S1: Experimental setup showing the arrangement of 16 settlement plates on horizontally (benign environment) and vertically (harsh environment) suspended backing panels in the field. Overall, I deployed a total of 20 backing panels in the field, 10 within each environment. Panels were deployed across two independent experimental runs.

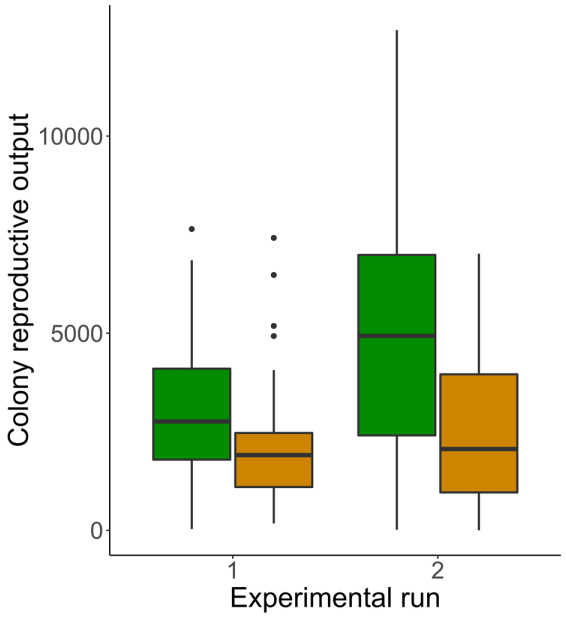

Figure S2: Boxplots depicting the distribution of colony reproductive outputs (cumulative number of ovicells) in the benign (green) and harsh (orange) environment, respectively, in each experimental run.

Table S1: The mean and variance (σ^2) in absolute fitness, and the opportunity for selection ($I \pm CI_{95\%}$) in the benign and harsh environment, respectively, in each experimental run.

	Mean	$\boldsymbol{\sigma^2}$	***N***	***I* (± $\mathbf{CI_{95\%}}$)**
Exp. run 1				
benign	2862.15	2715810.9	60	0.33 (0.2, 0.47)
harsh	2100.19	1908289.9	61	0.43 (0.26, 0.62)
Exp. run 2				
benign	4930.4	8883556.8	89	0.37 (0.26, 0.48)
harsh	2489.6	3661742.2	86	0.59 (0.41, 0.77)

Table S2: Repeated measures ANCOVA examining the effects of MI-MRE and environment on colony size over time in both experimental runs. Non-significant interactions were excluded from the final model (Table S3).

	MS	**numDF**	**denDF**	**F**	***P***
Between subjects					
experimental run	234.16	1	17	334.62	**< 0.0001**
MI-MR_E	0.17	1	275	0.25	0.62
environment	11.85	1	17	16.94	**0.0007**
residual	0.69				
Within subjects					
time	820.26	11	2652	2396.04	**< 0.0001**
time × experimental run	22.14	11	2652	64.68	**< 0.0001**
time × MI-MR_E	0.17	11	2652	0.49	0.91
time × environment	3.39	11	2652	9.91	**< 0.0001**
residual	0.34				

Table S3: Non-significant interactions excluded from the final model examining the effects of mass-independent metabolic rate early (MI-MR_E) and environment on colony size.

	MS	**numDF**	**denDF**	**F**	***P***
Between subjects					
MI-MR_E × environment	0.03	1	274	0.04	0.84
experimental run × MI-MR_E	0	1	273	0	0.99
experimental run × environment	0.13	1	16	0.19	0.67
experimental run × MI-MR_E × environment	0.01	1	272	0.01	0.91
Within subjects					
time × MI-MR_E × environment	0.13	11	2641	0.37	0.97
time × experimental run × MI-MR_E	0.11	11	2630	0.31	0.98
time × experimental run × environment	0.16	11	2619	0.47	0.93
time × experimental run × MI-MR_E × environment	0.26	11	2608	0.77	0.67

Chapter 4

Testing predictions of metabolic theory: does metabolic rate affect population demography in the field?

Abstract

Metabolic rate is thought to drive demography by determining the rate at which individuals use energy. Known as the 'energy equivalence rule', this mechanistic theory has long been invoked to explain classic relationships between body size and population density. Yet, body size affects demography via other life-history traits, so body size-density relationships provide only indirect support. A more direct test is to experimentally vary metabolic rate alone and examine its effects on population performance. I used a model marine colonial invertebrate, *Bugula neritina*, to create 172 experimental populations consisting of 1028 individuals of known metabolic rates and monitored their performance in the field. Contrary to expectations, higher metabolic rate populations had higher reproductive outputs during early stages, despite negative density-dependence, although the cumulative reproductive outputs (population yields) were unaffected by metabolic rate. I suspect that higher metabolic rate populations may have been able to extract relatively more resources from the environment, violating the assumptions of classic metabolic theory around population demography. My study suggests that classic relationships between body size and demography may not be driven by resource demands alone, but more field tests are necessary.

Introduction

Metabolic rate influences the rate at which individuals transform resources, use energy, and live (Hulbert and Else 2000, Pettersen et al. 2018), such that it may drive many ecological and evolutionary processes (Brown et al. 2004, but see Glazier 2015). Metabolic rate varies among species, among individuals of the same species, and populations (Burton et al. 2011, Konarzewski and Książek 2013, White and Kearney 2013). Although the consequences of this inter- and intraspecific variation in metabolic rate are increasingly well understood, I know little about the consequences for populations.

Theory predicts that metabolic rate should determine a population's carrying capacity by setting *per capita* resource demands (Brown et al. 2004, Calder 1984, Damuth 1981, 1987, Isaac et al. 2012). The idea that the carrying capacity of a population – the density at which a population stops growing and population productivity diminishes – is directly linked to individual metabolic rates has intuitive appeal. Because higher metabolic rates are associated with higher resource demands, a population's carrying capacity should decrease with increasing metabolic rate. Yet, the relationships between metabolic rate and population-level parameters are rarely tested directly. Instead, empirical support comes from indirect tests only, mostly in the form of among-species comparisons.

Among species, metabolic rate strongly covaries with body size (Calder 1984, Peters 1986). Most tests of how metabolic rate affects demography have been made by exploring how body size covaries with population density at carrying capacity, and whether this covariance matches predictions from metabolic theory (Damuth 1981, 1987, Hatton et al. 2015, Perkins et al. 2019). For example, because larger species have higher *absolute* metabolic rates, population density at carrying capacity should covary negatively with body size – and among species, they often do (Damuth 1981, 1987, Hatton et al. 2015, Perkins et al. 2019). Furthermore, because metabolic rate scales hypo-allometrically with body size in

metazoans (Brown et al. 2004, Kleiber 1932), larger species have lower *relative* metabolic rates, such that, per unit body mass, larger species have lower energy demands. Consequently, the total biomass of a population at carrying capacity should covary positively with body size (Blackburn and Gaston 1994, Maurer and Brown 1988). For example, because metabolic rate scales with body size at an exponent of around 0.75 in mammals (Brown et al. 2004), mammal population density should scale with body size at -0.75, and total biomass should scale with body mass at around 0.25 (Isaac et al. 2012). A corollary of these predictions is that total energy consumption of a population should be mass-independent, that is, two populations consisting of individuals differing in their mean body size should have equivalent energy consumption rates (Damuth 1981, 1987). Among-species comparisons of mass and population density have long been used as indirect (albeit very compelling) evidence for the negative relationship between energy consumption rates and density (Damuth 1981, 1987, Hatton et al. 2015, Perkins et al. 2019), and is sometimes known as the 'energy equivalence rule'.

Despite the often-remarkable congruence between the predicted and the observed relationship between body size and population density among species, this relationship does not directly test the link between metabolic rate and population demographic processes. Yet, the 'energy equivalence rule' and the links between body size and population processes are explicitly mechanistic – body size should affect population processes because of different relative metabolic rates. However, species of different body sizes also differ in myriad other life-history traits such as growth, longevity, and reproduction (Calder 1984), all of which also affect demography. Thus, the interspecific covariance between body size and demography could be driven by metabolic rate, but it could also be driven by potentially confounding factors (Tilman et al. 2004).

A more direct test of the link between metabolic rate and demography is to manipulate metabolic rate within species. In a rare and elegant example, Bernhardt et al. (2018) manipulated metabolic rate by growing phytoplankton under different thermal regimes. Their results were consistent with theoretical expectations based on among-species comparisons: population density at carrying capacity decreased with increasing metabolic rates at higher temperatures (see also Reuman et al. 2014). However, beyond manipulations of temperature, which can also influence other factors beyond metabolic rate, few studies vary metabolic rate in isolation, and examine its consequences on demographic processes, particularly under field conditions. I would argue that such tests are critical because alternative metabolic theory makes conflicting predictions about how metabolic rate might affect energy acquisition and demography.

Theories about metabolic rate that focus on different scales of organisation (i.e., population-level or individual-level) make conflicting assumptions about energy acquisition and use. Since its inception, discussions regarding the 'energy equivalence rule' and other metabolic theories at the population level have made the simplifying assumption that the rate of resource delivery into a population is independent of metabolic rate or body size (Maurer and Brown 1988, Nee et al. 1991). On the other hand, metabolic theories based on studies of individuals assume that metabolic rate affects access to resources. For example, the 'increased-intake' hypothesis predicts that individuals with a higher metabolic rate have faster physiologies, which may allow them to forage more voraciously or effectively, such that they can extract more resources from their environment (Biro and Stamps 2010, Chappell et al. 2007, McNab 1980). Thus, resource supply into a population could positively covary with metabolic rate – a conclusion that contradicts the assumptions of energy equivalence. The way in which the 'increased-intake' hypothesis affects population-level demography, however, remains unclear.

Although the 'increased-intake' hypothesis has empirical support from studies of individuals, it could be that populations consisting of higher metabolic rate individuals simply access and deplete available resources faster, without affecting the actual supply of resources. In this case, the predictions of the 'increased-intake' hypothesis would coincide with those of the 'energy equivalence rule' – high metabolic rate populations would have lower carrying capacities. Alternatively, if metabolic rate alters the effective supply of resources into a population – for example, by exploiting passing resources more effectively or accessing different resource pools – then the 'increased-intake' hypothesis would predict the opposite pattern – high metabolic rate populations should have higher carrying capacities. Despite intense, long-standing interest in the links between metabolic rate and demography, and the differing predictions about the nature of such links, I am aware of none that experimentally manipulate the metabolic rate alone of whole populations and examine its consequences.

Here, I test predictions of different metabolic theories by creating experimental populations of differing densities with different *per capita* metabolic demands. I took advantage of the natural and persistent variation in metabolic rate among *Bugula neritina* individuals (Pettersen et al. 2016, Pettersen et al. 2020, Schuster et al. 2019) and their sessile nature to create populations that systematically differed in their metabolic rates. I deployed these populations into a common environment in the field, with all individuals within the populations being of equivalent size and age. I then monitored the growth and reproduction of all individuals across their lifetime to estimate how metabolic rate and population density affect the biomass yield and reproductive outputs of whole populations.

Materials and methods

Study species, site and field deployment

Bugula neritina Linnaeus, 1758, is common to sessile marine communities worldwide. Colonies grow by asexual budding of new zooids (individual subunits) at the distal ends combined with regular bifurcations after approximately every four pairs of zooids to produce symmetrical branching (Keough and Chernoff 1987, Keough 1989). Once colonies reach sexual maturity, zooids develop clearly visible brood structures called ovicells (Woollacott and Zimmer 1975). Each ovicell broods a single larva, which is released into the plankton after approximately one week once embryogenesis is complete. Upon release, the non-feeding larvae are immediately competent to settle.

I collected sexually mature *B. neritina* colonies from the Royal Melbourne Yacht Squadron in Port Phillip Bay, Victoria, Australia (-37.865, 144.966), in April 2018. To obtain individuals for my experiment, I spawned colonies according to standard procedures (Marshall et al. 2003). Briefly, I maintained colonies in the laboratory in the dark for 48 h, after which I induced them to spawn by exposing them to bright light. I settled single larvae in a drop of seawater onto roughened A4 acetate sheets to induce settlement (~ 200 settlers per sheet). After three hours, I rinsed any unsettled larvae from the acetate sheets and kept settlers in tanks with unfiltered seawater. The following day, I deployed the acetate sheets bearing settlers into the field by attaching them to PVC backing panels (57 × 57 × 0.6 cm). I deployed a total of eight panels, with each panel bearing three acetate sheets. I suspended the panels 1 m below the water surface with settlers facing down at the Royal Brighton Yacht Club (-37.909, 144.986). I conducted collections of colonies, spawning, and field deployments across two weeks.

Metabolic rate measurements and creating populations

After each panel had been in the field for two weeks (bearing two weeks old colonies), I returned the acetate sheets to the laboratory to conduct metabolic rate measurements. I maintained colonies in aerated tanks with field-collected seawater at 19°C overnight. Prior to metabolic rate measurements, I removed any epibionts and debris from the colonies. I then separated individual colonies from the A4 sheets by cutting around the base of the colonies such that each colony was attached to a small square of acetate sheet. I placed individual colonies into 750 µl glass vials containing pasteurized, 0.2 µm filtered seawater and a non-consumptive O_2 sensor spot (Loligo Systems, Denmark). I then measured oxygen consumption rates (as $\dot{V}O_2$) as per standard techniques (Schuster et al. 2019). To convert $\dot{V}O_2$ (millilitres per hour) to metabolic rate (milliJoules per hour), I used the calorific conversion factor of 20.08 J ml^{-1} O_2 (Crisp 1971). Following metabolic rate measurements, I counted the number of zooids of each colony as a proxy for body mass. For *B. neritina*, the number of zooids and body mass are strongly correlated (Schuster et al. 2019). Colonies used for metabolic rate measurements ranged from 28 to 124 zooids in size (two to four bifurcations).

Since metabolic rate covaries positively with body size, and mass-specific metabolic rate covaries negatively with body size, I could not use either estimate for metabolic rate – such an estimate would be a proxy for body size. Instead, I calculated mass-independent metabolic rates (MI-MR), a more reliable estimate of individual metabolic rate for a given body size. I estimated MI-MR by using regression of metabolic rate on body size (nonlinear regression of the form $MR = a * M^b$, where MR is metabolic rate, M is colony mass, a is the intercept, and b is the scaling exponent) and extracting the residuals – a positive residual indicates that an individual has a higher-than-average metabolic rate for its size, a negative residual indicates the opposite.

I then created experimental populations that consisted of individuals of known MI-MRs. I glued either four or eight individuals onto 25 cm^2 PVC plates (55 × 55 × 3 mm). These densities overlap with densities typically observed in the field (field observations, 0–0.75 colonies/cm^2 (Hart and Marshall 2013); densities in this experiment, 0.16 and 0.32 colonies/cm^2). For simplicity, I refer to my two densities as either 'low density' (four individuals per 25 cm^2) or 'high density' (eight individuals per 25 cm^2) hereafter. I deliberately varied mean mass-independent metabolic rates of my experimental populations to create a continuous range of metabolic rates (hereafter Mean Population MR). Mean *absolute* population metabolic rates (the average of *absolute* individual metabolic rates; ± SD) varied between 3.49 ± 0.7 mJ h^{-1} and 18.95 ± 4.64 mJ h^{-1} in low-density populations, and between 3.51 ± 0.52 mJ h^{-1} and 23.21 ± 3.74 mJ h^{-1} in high-density populations. Overall, there was a 5.43-fold difference in *absolute* metabolic rates between the lowest and the highest metabolic rate populations at low density, and a 6.61-fold difference in metabolic rate between the lowest and the highest metabolic rate populations at high density. Because colonies were attached to a square of acetate sheet, I additionally glued four empty squares of acetate sheet onto plates with four individuals (low density), so that all plates had a total of eight squares of acetate sheet. I deployed the experimental populations (my unit of replication) into the field at the Royal Brighton Yacht Club by attaching them to PVC panels as previously described. I deployed a total of 172 experimental populations consisting of 1028 individuals. 87 of my experimental populations were low-density populations, and 85 were high-density populations.

Monitoring population-level performance

I measured the performance of my experimental populations by monitoring the survival, growth, and reproduction of all individuals every two weeks for their entire life history (i.e.

until all individuals had died; April through to October 2018). Throughout the experiment, I removed any dead colonies from my experimental populations. I measured growth as the number of bifurcations as an indication of colony size (Keough and Chernoff 1987) and reproduction as the number of ovicells contained on each individual at each census. I then summed the total number of ovicells on each individual across their lifetime and summed these across all individuals within each experimental population to derive the cumulative reproductive output of each population.

I also measured the size of second-generation offspring produced by the colonies within my experimental populations after 16 weeks in the field (when fecundity of my experimental populations was highest). To do this, I spawned my experimental populations in separate containers using the methods described above. I fixed all larvae released by each experimental population in separate vials containing 3.5% formalin-seawater solution, which does not distort larval size (Marshall et al. 2003). I stored the preserved larvae in these conditions for up to three months. Where possible, I measured 50 larvae per experimental population from photographs according to standard techniques (Marshall et al. 2003). I then calculated the mean offspring size (µm) for each experimental population and subsequently converted offspring size to offspring mass (µg) using an equation that describes this relationship (Pettersen et al. 2015).

Monitoring changes in population-level metabolic rates

I was interested in whether the differences in population metabolic rate would persist over time, i.e. whether populations with initially higher metabolic rates would also have higher metabolic rates later on. Thus, I returned my experimental populations to the laboratory after eight weeks in the field. Prior to metabolic rate measurements, I acclimated my populations in field-collected seawater at 19 °C overnight. I used 1L acrylic hermetic water baths, each

connected to a peristaltic pump to ensure continuous water flow (as described in Ghedini et al. 2017). I measured 12 populations at a time (one population per water bath) and included two control baths in each measurement run whereby I placed one PVC plate in each bath that had eight empty acetate squares glued onto them. I deployed these control plates together with my experimental populations in the field (four control plates per panel) and removed any settled animals prior to metabolic rate measurements. I recorded $\dot{V}O_2$ in a constant-temperature room at 19°C over 3h using an optical oxygen meter (FireStingO2, PyroScience GmbH, Germany) and flow-through cells containing an oxygen sensor (PyroScience GmbH, Germany). I then calculated total population metabolic rates (Population Total MR) as per White et al. (2011). Following metabolic rate measurements, I counted the number of zooids of each colony as an approximation of population biomass (the number of zooids of each colony summed within each population).

Statistical analysis

My main objective was to test for the effects of Mean Population MR, population density, and their interaction on the performance of my experimental populations (i.e. population yield) in the field. To do so, I conducted repeated measures analyses of covariance (RM ANCOVA) with density and time (measurement points) as categorical fixed effects, Mean Population MR as the covariate of interest, and the mean *per capita* reproductive output or mean colony size, respectively, as the response variable. I further used a linear model to test for the effects of Mean Population MR and density on the cumulative reproductive outputs (i.e. population yields) after six months.

To test for the effects of Mean Population MR and density on late survival (after 24 weeks in the field), I used a binomial generalized linear model (GLM) with a log-it function, weighted by the number of individuals within each population. I also tested for the effects of

Mean Population MR, $\log_{10}$Population biomass, and their interaction on Population Total MR. Since the population biomasses of low- and high-density populations differed, I conducted two separate analyses for both population densities due to non-overlapping covariance ranges.

In all analyses, I included panel (my level of replication) as a fixed effect. I reduced each model by removing non-significant interactions if their inclusion did not improve the model fit (Quinn and Keough 2002). I conducted all statistical analyses in R version 3.6.2 (R Core Team 2017) using the packages *lmerTest* (Kuznetsova et al. 2017) and *car* (Fox and Weisberg 2019).

Results

Population reproductive output and growth

The effect of mean population metabolic rates on the reproductive outputs of my experimental populations changed over time (significant Mean Population MR × time interaction; Table S1). The interaction was driven by high metabolic rate populations having higher reproductive outputs at 12 weeks, before these differences dissipated (Fig. 1). Nonetheless, I could not detect a significant effect of Mean Population MR on the cumulative reproductive output (i.e. population yields) of my experimental populations after six months in the field ($F_{1,162} = 2.66$, $P = 0.11$; Fig. 2). Population density did not alter the effect of Mean Population MR ($F_{1,161} = 0.06$, $P = 0.81$), but high-density populations had lower *per capita* reproductive outputs overall ($F_{1,162} = 47.16$, $P = 1.33 \times 10^{-10}$). At high density, individuals produced on average 3470.73 (± 136.66 SE) offspring throughout their life, while at low density the mean *per capita* reproductive output was 4743.13 (± 213.18 SE) offspring.

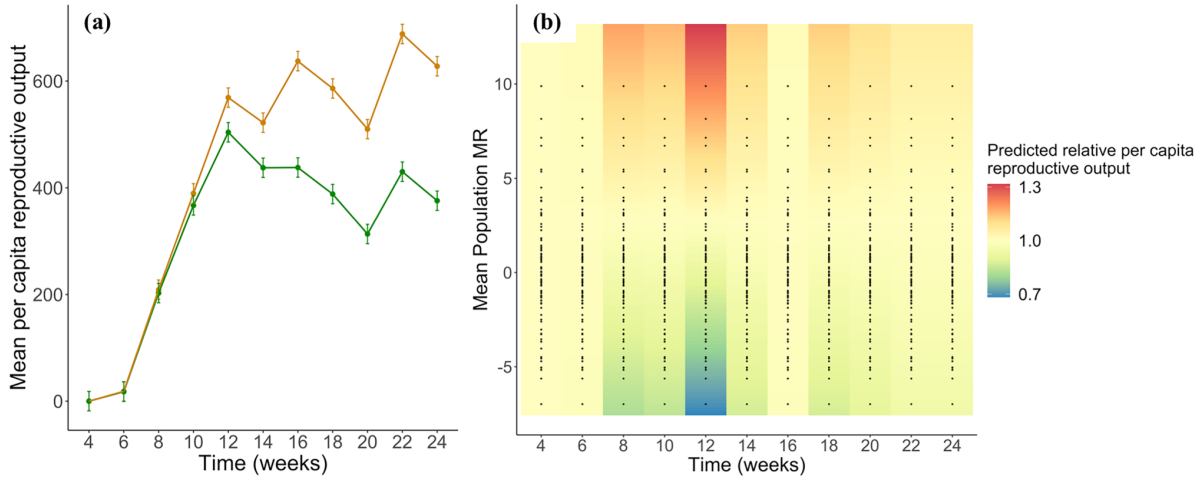

Figure 1: The mean *per capita* reproductive output of low- (orange line) and high-density (green line) populations plotted against time (in weeks) (a), and the effect of Mean Population MR on the relative *per capita* (standardized to the mean) over time (in weeks) at both densities (b). Error bars show standard errors (a). Black dots show the underlying data points (b).

Colony size was not affected by Mean Population MR and the effect did not change over time (Table S2), indicating that the differences in reproductive output at 12 weeks were not driven by differences in colony size. Population density, however, negatively affected the size of individuals, and the effect strengthened over time (Table S2, Fig. 3). Individuals in high-density populations were on average 5% smaller in terms of bifurcations than individuals in low-density populations at the end of the experiment. Furthermore, mean offspring size was neither affected by Mean Population MR ($F_{1,162} = 0.64$, $P = 0.43$) nor density ($F_{1,162} = 1.08$, $P = 0.29$), although high-density populations tended to produce larger offspring (mean ± SE; low density: 265.5 µm ± 1.2; high density: 267.7 µm ± 1). The effect of Mean Population MR on mean offspring size did not differ between densities ($F_{1,161} = 0$, $P = 0.99$). Hence, high metabolic rate populations did not increase their reproductive outputs by producing more but smaller offspring at 12 weeks.

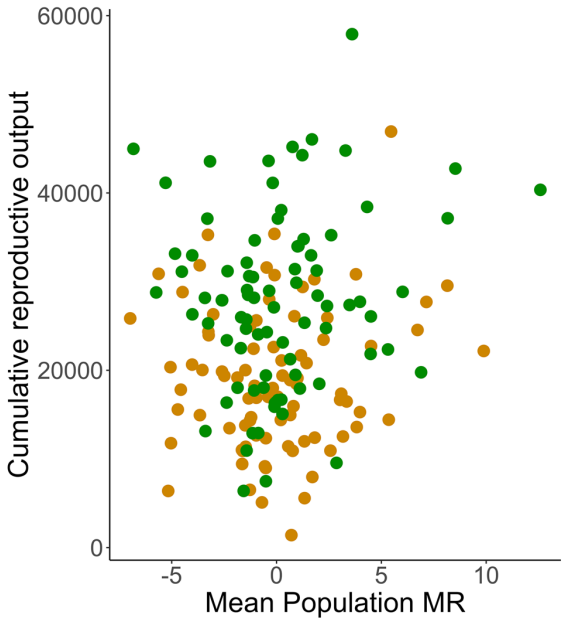

Figure 2: The cumulative reproductive outputs (population yields) of low- (orange dots; N = 87) and high-density (green dots; N = 85) populations plotted against Mean Population MR.

Since I could not detect a significant effect of Mean Population MR on colony size, I calculated size-specific reproductive outputs by dividing the reproductive output by the total number of zooids (i.e. biomass) of each population at 12 weeks. Density did not affect the size-specific reproductive output ($F_{1,162} = 1.85$, $P = 0.18$), but there was a positive effect of Mean Population MR ($F_{1,162} = 15.85$, $P = 0.0001$). The size-specific reproductive output increased with Mean Population MR, indicating that for any given population biomass, higher metabolic rate populations had greater size-specific reproductive outputs (Fig. S1). The effect of Mean Population MR did not differ between densities ($F_{1,163} = 0.58$, $P = 0.45$).

Survival

Early survival was high up to 22 weeks, ranging between 50% and 100%, but almost all populations had 100% survival (survival at 22 weeks, mean = 98.11% ± 0.54 SE). At 24 weeks, mean individual survival decreased to 77% (± 2.12 SE). Here, survival was affected

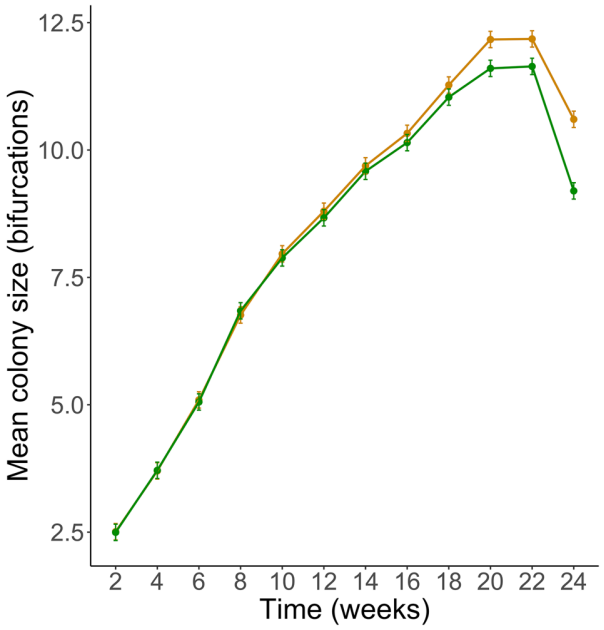

Figure 3: Mean colony size (as mean number of bifurcations) in low- (orange line) and high-density (green line) populations plotted against time (in weeks). Error bars show standard errors.

by population density ($\chi^2 = 9.28$, $df = 1$, $P = 0.002$), with survival being higher in low-density populations (survival at low density: mean = 80.75% ± 0.27 SE; at high density: mean = 73.24% ± 0.27 SE). Mean Population MR did not affect survival at 24 weeks ($\chi^2 = 0.02$, $df = 1$, $P = 0.89$), and the effect did not differ between population densities ($\chi^2 = 0.82$, $df = 1$, $P = 0.37$).

Population metabolic rates

After eight weeks in the field, I could not detect a significant effect of Mean Population MR on Population Total MR at either density (low density: $F_{1,77} = 0.88$, $P = 0.35$; high density: $F_{1,75} = 1.01$, $P = 0.32$). I also could not detect a significant Mean Population MR × population biomass interaction (low density: $F_{1,76} = 2.67$, $P = 0.11$; high density: $F_{1,74} = 0.08$,

$P = 0.78$), whereas population biomass had a strong effect on Population Total MR (low density: $F_{1,77} = 14.92$, $P = 0.0002$; high density: $F_{1,75} = 6.09$, $P = 0.02$).

Discussion

Populations consisting of high metabolic rate individuals had higher reproductive outputs during early stages, whereas the cumulative reproductive outputs (i.e. population yields) were not affected by population metabolic rates. Current metabolic theory based on among-species comparisons predicts that a population's carrying capacity should decline with increasing metabolic rates (Damuth 1981, 1987, Hatton et al. 2015, Perkins et al. 2019) because of increasing *absolute* resource demands (Brown et al. 2004). When metabolic rate varies but body size is kept constant among populations of the same species, I find that metabolic rate affects population-level performance in a way that is opposite to that predicted by classic theory. Hence, my results provide support for predictions based on how metabolic rate affects access to resources rather than resource demands alone (Burton et al. 2011).

My findings that *absolute* metabolic rate does not drive demography are foreshadowed by studies that explore metabolic rate and demography across a wider range of body size. For example, negative correlations between body size and density are not found across species when comparisons are made within taxonomic groups (Isaac and Carbone 2010). In the past, a lack of an effect across more narrow body size ranges has been attributed to a lack of statistical power (Isaac et al. 2012). Instead, it might be that comparisons within narrow taxonomic groups mean that the differences in life-history traits are minimised and it is these traits, rather than metabolic rate, that were driving the patterns at higher taxonomic levels. Similarly, when body size is experimentally evolved to be different, size does not covary consistently with population-level demography as predicted by metabolic theory (Malerba and Marshall 2019).

The lack of congruence between the predictions of metabolic theory and my results surprised me because it is reasonable to expect that metabolic rate imposes a maximum limit on population size for a given resource level (Isaac et al. 2012). My experimental populations were below carrying capacity and so I did not sample the absolute maximum population density possible in my system. Nonetheless, I find strong density effects on population performance indicating that my higher density was approaching carrying capacity, resulting in limited *per capita* access to resources and reduced individual performance (Antonovics and Levin 1980, Ghedini et al. 2017, Violle et al. 2010). If resource supply had stayed constant, then I should have observed a negative relationship between metabolic rate and performance in the high-density populations as these would have had the greatest resource demands.

Overall then it seems that metabolic rate does not directly affect population-level processes by simply setting total resource demands alone. I suspect that higher metabolic rate populations accessed more resources from the environment in my experiment. In classic metabolic theory, resource supply is kept constant and is unaffected by either body size or metabolic rate (Damuth 1981, 1987, Maurer and Brown 1988, Nee et al. 1991). In a recent laboratory-based study, Bernhardt et al. (2018) explored the role of temperature-mediated differences in metabolic rate driving carrying capacity and found strong support for classic metabolic theory, i.e. a negative linear relationship between carrying capacity and metabolic rate. Under field conditions, however, access to resources can vary naturally. Furthermore, resource supply into a population might be affected through correlations between metabolic rate and other traits that determine resource intake. For example, individuals with higher metabolic rates may increase their resource intake through increased feeding activity, as previously shown in a range of taxa (reviewed in Biro and Stamps 2010). When it becomes possible for metabolic rate to affect resource supply, the expectations based on classic theory

are no longer met. This idea requires further testing but it seems to me at least that theory around the effects of metabolic rate on resource intake at an individual level (e.g., the 'increased-intake' hypothesis, Burton et al. 2011) needs to be applied to classic theory around the effects of metabolic rate at the population level.

A higher reproductive output early in life has important implications for demographic processes across generations. Given that higher reproduction early on is linked to a shorter generation time, high metabolic rate populations will have higher population yields than lower metabolic rate populations across generations. Nevertheless, the covariance between metabolic rate and individual fitness, i.e. selection on metabolic rate, can vary both in space and time (Nilsson and Nilsson 2016, Robertsen et al. 2014), thereby maintaining variation in metabolic rate overall. For example, it may be that certain times of the year or conditions favour lower metabolic rates, thereby maintaining variation in metabolic rate overall. Alternatively, selection on metabolic rate may be frequency-dependent – I kept the metabolic rates within my populations relatively similar; it would be interesting to determine how a low metabolic rate individual performs in a high metabolic rate population and vice versa.

Although I found that high metabolic rate populations had higher reproductive outputs early on, why did I not find an overall positive relationship between metabolic rate and population yields? Metabolic rate is linked to a range of life-history traits and is therefore a main driver of the pace of life (Pettersen et al. 2016, Pettersen et al. 2018). For example, individuals with higher metabolic rates often have faster life histories, resulting in higher growth rates, earlier reproduction, and shorter lifespans. Despite the fact that I did not find any differences in longevity across metabolic rates, populations consisting of high metabolic rate individuals might have experienced higher rates of reproductive senescence in later stages, leading to overall similar reproductive outputs across metabolic rates.

Metabolic rate is generally repeatable, but repeatability declines over time (White et al. 2013), especially under field conditions (Auer et al. 2016). After eight weeks in the field, I could not detect a signal of initial Mean Population MR on Population Total MR, suggesting an overall low repeatability of metabolic rate at the population level. Population density can affect both access to resources (Ghedini et al. 2017) and oxygen availability within a population (Lagos et al. 2017), resulting in metabolic suppression. Given that I found a strong density effect on population performance, resulting in reduced *per capita* reproductive outputs and smaller mean colony sizes, my experimental populations likely reached similar metabolic rates due to metabolic suppression. Nonetheless, I would have expected to find a stronger effect of metabolic suppression at high density, given that access to resources and oxygen availability are more limited at higher population densities (Ghedini et al. 2017, Lagos et al. 2017). Why I did not find a difference in repeatability between densities remains unclear to me at this point.

The repeatability of metabolic rate at the population level might be low, but an individual's metabolic rate early in life may still affect performance later on. For example, as previously shown in a range of species, higher metabolic rate promotes higher feeding rates and energy intake (Biro and Stamps 2010), but a higher metabolic rate might also be linked to a higher resource storage. As density effects strengthen over time and the *per capita* access to resources becomes more and more limiting, stored resources may become crucial for maintaining a high energy turn-over, thereby increasing reproductive outputs. In addition, a higher metabolic rate might also be linked to greater immunity, making these individuals less prone to diseases and infections. Hence, even though an individual's metabolic rate changes over time, having a higher metabolic rate early in life might still have fitness advantages later on.

For *B. neritina*, Allen et al. (2008) have recently shown that individuals produce larger offspring at higher population densities, whereas I did not find an effect of population density on offspring size. Compared to their study, however, the differences between the low- and high-density treatments were much more subtle in my study. Although the low-density treatments were similar in both studies (0.16 individuals/cm^2), the high-density treatments differed considerably (my study: 0.32 individuals/cm^2; Allen et al. (2008): 0.63 individuals/cm^2). Thus, I simply may not have been able to detect strong effects of population density on offspring size, although individuals in my high-density treatment tended to produce slightly larger offspring.

Ecologists have long linked demographic processes at the population level to differences in metabolic rate, but classic theory is mostly based on among-species comparisons (Damuth 1981, 1987, Hatton et al. 2015, Perkins et al. 2019). In what I suspect is the first study of its kind, I show that within a species metabolic rate does not affect population demographic processes as anticipated by metabolic theory. Rather, metabolic rate likely not only sets resource demands (Brown et al. 2004), but also affects resource supply into or resource intake within a population (Burton et al. 2011). Thus, I encourage further tests where populations of known metabolic rate are followed under field conditions or at least allow resource supply to naturally covary with metabolic rate. Furthermore, in light of my findings, I propose that resource intake theory be considered when predicting metabolic rate-demography relationships at the population level.

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Supplementary material

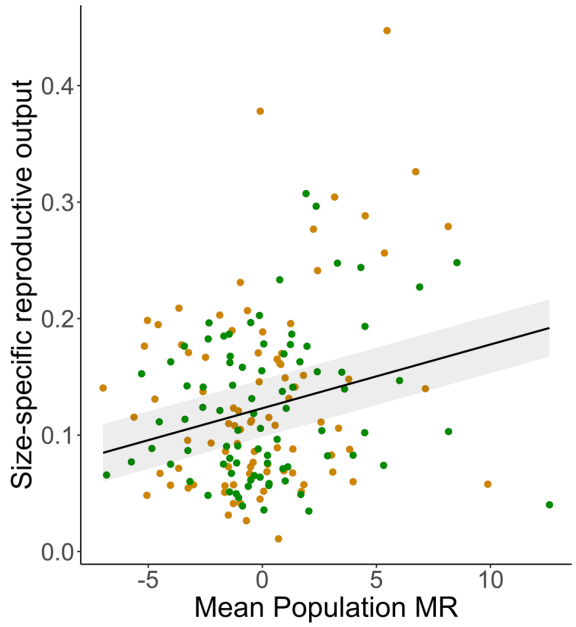

Figure S1: Predicted line of best fit from linear models (with 95% C.I.) for the relationship between size-specific reproductive output and Mean Population MR. Orange dots are low-density populations; green dots are high-density populations. Size-specific reproductive output was calculated by dividing the reproductive output by the total biomass (number of zooids of each colony summed up across individuals within a population) at 12 weeks for each population. Data points represent observed values for each population.

Table S1: Repeated measures ANCOVA examining the effects of Mean Population MR, density, and panel on the mean *per capita* reproductive outputs over 24 weeks in the field. Non-significant interactions were removed from the final model (Table S3).

	MS	**df**	**F**	***P***
Between subjects				
Panel	121687	7	6.49	$\mathbf{9.35 \times 10^{-7}}$
Density	645583	1	34.45	$\mathbf{2.39 \times 10^{-8}}$
Mean Population MR	39633	1	2.12	0.15
Residual		162		
Within subjects				
Time	6956060	10	371.24	$\mathbf{< 2.2 \times 10^{-16}}$
Time × panel	266625	70	14.23	$\mathbf{< 2.2 \times 10^{-16}}$
Time × density	496957	10	26.52	$\mathbf{< 2.2 \times 10^{-16}}$
Time × Mean Population MR	45693	10	2.44	**0.007**
Residual		1617		

Table S2: Repeated measures ANCOVA examining the effects of Mean Population MR, density, and panel on mean colony size (bifurcations) over 24 weeks in the field. Non-significant interactions were removed from the final model (Table S3).

	MS	**df**	**F**	***P***
Between subjects				
Panel	10.49	7	19.94	**$< 2.2 \times 10^{-16}$**
Density	4.96	1	9.43	**0.003**
Mean Population MR	0.02	1	0.04	0.83
Residual		162		
Within subjects				
Time	1544.4	11	2936.52	**$< 2.2 \times 10^{-16}$**
Time × panel	14.5	77	27.57	**$< 2.2 \times 10^{-16}$**
Time × density	6.46	11	12.28	**$< 2.2 \times 10^{-16}$**
Time × Mean Population MR	0.14	11	0.27	0.99
Residual		1779		

Table S3: Non-significant interactions excluded from the final model examining the effects of Mean Population MR, density, and panel on (a) the *per capita* reproductive output and (b) mean colony size (bifurcations) over 24 weeks in the field.

	MS	**df**	**F**	***P***
(a) mean *per capita* reproductive output				
Between subjects				
Density × Mean Population MR	1634	1	0.09	0.77
Within subjects				
Time × density × Mean Population MR	6698	10	0.36	0.96
(b) mean colony size				
Between subjects				
Density × Mean Population MR	0.23	1	0.43	0.51
Within subjects				
Time × density × Mean Population MR	0.19	11	0.36	0.97

Chapter 5

Metabolic phenotype mediates the outcome of competitive interactions in a response-surface field experiment

Abstract

Competition and metabolic rate are tightly linked. Within a population, individuals vary in their metabolic rates and such differences in resource demands are often associated with different competitive abilities. The effects of metabolic rate on conspecific interactions, however, have mostly been studied under laboratory conditions. By using a trait-specific response-surface design, I tested for the effects of metabolic rate on pairwise interactions of the marine colonial invertebrate, *Bugula neritina* in the field. Specifically, I compared the performance (survival, growth, and reproduction) of focal individuals, both in the presence and absence of a neighbour colony, both of which had their metabolic phenotype characterised. Survival of focal colonies depended on the metabolic phenotype of the neighbouring individuals, or on the combination of both the focal and neighbour colony metabolic phenotypes that were present. I found that low metabolic rate individuals always grew more than high metabolic rate individuals. Surprisingly, I found pervasive effects of neighbour metabolic phenotypes on focal performance, though the sign and strength of these effects varied in space. Neighbouring colonies with lower metabolic rates (that grew more) always affected the reproductive output of focal colonies but both persistence and direction of this affect varied. Overall, I find that the metabolic phenotype changes the strength of competitive interactions, but these effects are highly contingent on local conditions. I suggest future studies explore how variation in metabolic rate affects organisms beyond the focal organism alone, particularly under field conditions.

Introduction

An individual's metabolic rate and competitive ability are tightly linked (Sloman and Armstrong 2002, Biro and Stamps 2010b). Competition for resources among and within species constrains the acquisition of energy by individuals and, consequently, their growth and fitness. An individual's metabolic rate is tightly linked to its resource demands, with individuals with higher metabolic rates having greater resource requirements (Burton et al. 2011, Brown et al. 2004). Especially at high population densities, competition limits *per capita* resource availability (exploitative competition) or access to resources (interference competition) (Antonovics and Levin 1980, Violle et al. 2010). Accordingly, individuals sometimes downregulate their metabolic rates in response to intraspecific competition so as to maintain positive energy fluxes despite lower resource availability (DeLong et al. 2014, Ghedini et al. 2017). Yet competitive environments do not always favour lower, 'more thrifty' metabolic phenotypes.

Higher metabolic rate individuals can increase their competitive ability by increasing access to, or use of, scarce resources due to their higher activity levels, greater boldness, territorial aggression and competitive dominance (Careau et al. 2008, Biro and Stamps 2010b). Similarly, metabolic rate is known to covary with a range of traits that can influence resource acquisition – individuals with higher metabolic rates may forage more voraciously or effectively (McNab 1980, Chappell et al. 2007, Biro and Stamps 2010b). Consequently, individuals with higher metabolic rates may be able to gain preferential access to resources or extract disproportionately more resources from the environment (Pettersen et al. 2020), potentially resulting in asymmetric competition between metabolic phenotypes (Weiner 1990). To date, competitive interactions among conspecifics of varying metabolic phenotypes have mostly been studied under laboratory conditions where conditions are less variable

(Ward et al. 2006). However, if we are to understand how metabolic rate affects competition under natural, more variable conditions, field studies are a necessary next step.

The competitive advantages conferred by any one metabolic phenotype should depend strongly on environmental conditions. A higher metabolic rate is often associated with a higher energy turnover that can be beneficial for growth and survival when resource availability is high but disadvantageous when resource levels are low (Bochdansky et al. 2005, Armstrong et al. 2011, Burton et al. 2011, Killen et al. 2011, Auer et al. 2015, Auer et al. 2020). Individual differences in metabolic rate and associated traits such as body size may therefore interact with environmental conditions to determine the outcome of competitive interactions among conspecifics. For example, individuals with higher metabolic rates may only be able to grow larger and, therefore, be competitively dominant if the *per capita* resource levels are high (Ward et al. 2006). Conversely, higher metabolic rates may be disadvantageous in resource-limited environments if resources are simply insufficient to sustain individuals with higher energy demands (Auer et al. 2020). How environmental conditions interact with metabolic rate to determine competitive outcomes in the field, however, remains poorly understood.

In a manipulative field experiment, I examined how metabolic rate mediated conspecific interactions in the bryozoan, *Bugula neritina,* a colonial, sessile marine invertebrate. Colonies of *B. neritina* are commonly found as part of fouling communities throughout the world where they form dense congregations of conspecifics, suggesting that intraspecific competition may be particularly intense. I took advantage of the natural and persistent variation in metabolic rate among individual *B. neritina* colonies (Pettersen et al. 2016, Schuster et al. 2019, Pettersen et al. 2020) to test for the effects of metabolic rate on competition. I used a trait-specific response-surface design to create pairwise interactions of individuals with differing metabolic phenotypes (Inouye 2001, Cameron et al. 2019). Due to

the sessile nature of *B. neritina*, I was able to follow the performance of individuals across their entire adult lives in the field. I then measured the outcomes of pairwise interactions by comparing the performance (survival, growth, and lifetime reproductive output) of individuals with different metabolic rates, both in the presence and absence of a neighbour colony.

Materials and methods

Study species, site and field deployment

Bugula neritina Linnaeus, 1758, is an arborescent bryozoan common to sessile marine communities worldwide. *B. neritina* grow by asexual budding of connected zooids (individual subunits) at the distal end to produce symmetrical branching colonies (Keough and Chernoff 1987, Keough 1989). Once sexually mature, colonies form clearly visible structures called ovicells (Woollacott and Zimmer 1975). Each ovicell broods a single larva, which is released into the plankton once embryogenesis is complete. Upon release, the non-feeding larvae are immediately competent to settle, and most larvae settle within hours under field conditions (Burgess and Marshall 2011). Larvae also preferentially settle close to conspecifics in the laboratory (Keough 1984), and such aggregations of *B. neritina* conspecifics are often observed in the field.

I collected sexually mature *B. neritina* colonies from the Royal Melbourne Yacht Squadron in Port Phillip Bay, Victoria, Australia (-37.865, 144.966) in April 2019. To obtain individuals for my experiments, I spawned colonies according to standard procedures (Schuster et al. 2019). Briefly, I kept colonies in the laboratory in field-collected seawater in aerated tanks in the dark. After 48h, I spawned colonies by exposing them to bright light and settled single larvae in a drop of seawater on roughened A4 acetate sheets to induce settlement (~150 settlers per acetate sheet). After three hours, I rinsed unsettled larvae from

the acetate sheets and kept settlers in tanks with unfiltered seawater. The next day, I attached the A4 acetate sheets bearing settlers to five PVC backing panels (57 × 57 × 0.6 cm; two acetate sheets per panel) and suspended the panels 1 m below the water surface with settlers facing down at the Royal Brighton Yacht Club (-37.909, 144.986).

Mass-independent metabolic rate

To conduct metabolic rate measurements, I returned acetate sheets bearing settlers to the laboratory after they had been in the field for two weeks. I kept colonies in aerated tanks with field-collected seawater at 19°C overnight. Prior to metabolic rate measurements, I removed any epibionts and debris from the colonies. I then separated individual colonies from the A4 sheets by cutting around the base of the colonies such that each colony was attached to a small square of acetate sheet. In total, I measured the metabolic rates of 372 colonies with 750 µl glass vials (Loligo Systems, Denmark) at 19 °C as described in Schuster et al. (2019).

I estimated mass-independent metabolic rates (MI-MR) by regressing metabolic rate on colony mass (nonlinear regression of the form $MR = a * M^b$, where *MR* is metabolic rate, *M* is colony mass, *a* is the intercept, and *b* is the scaling exponent) and extracting the residuals. To determine colony size, I counted the number of zooids in each colony. Given that colonies were attached to squares of acetate sheet, zooid counts were more reliable than weighing them, and the number of zooids and colony mass are strongly correlated (Schuster et al. 2019). Colonies used for metabolic rate measurements ranged from 16 to 48 zooids in size.

Experimental design and field deployment

My main goal was to investigate whether metabolic rate mediates the outcome of pairwise interactions using a trait-specific, response-surface design (Inouye 2001, Cameron et al.

2019). I used the continuous range of metabolic rates from my source population (1.25 – 7.67 mJ h^{-1} *absolute* metabolic rates) to generate pairwise combinations of metabolic rates (maximum absolute differences in metabolism between pairs was 5.08 mJ h^{-1}; Fig. S1). To create my treatments, I glued two acetate sheet squares, each bearing a single colony, onto PVC plates (55 × 55 × 3 mm) at a distance of 1 cm from each other. I treated both these colonies as the focal colony and neighbour colony to test for reciprocal interactions (Inouye 2001). In addition, I estimated the baseline relationship between metabolic rate and performance of single colonies without a neighbour colony by gluing a blank acetate sheet square 1 cm from a focal colony. I then distributed a total of 162 plates across the five backing panels and redeployed them into the field. Note that I assigned plates haphazardly to each panel; consequently, there were no differences in colony sizes or MI-MRs among panels (zooids: $F_{4,260} = 0.44$, $P = 0.78$; MI-MR: $F_{4,260} = 0.08$, $P = 0.99$).

I followed the performance of 265 colonies of known metabolic rates throughout their entire life history, until all colonies had died (April through to October 2019). I followed the survival, growth, and reproductive output of each colony every two weeks. Colonies were considered alive if they were still attached to the plate and >10% of the colony contained feeding zooids. I measured the reproductive output of each colony by counting the number of ovicells throughout the duration of the experiment, and growth as the number of bifurcations at each measurement point (Keough and Chernoff 1987). I also removed any non-experimental settlers (both *Bugula* and other species) from the plate at each measurement point to eliminate competition from other organisms. Furthermore, to avoid any environmental effects associated with a colony's position within a panel on metabolic rates or performance, I moved each plate to a different position within the assigned panel every two weeks (Mitchell-Olds and Shaw 1987, Rausher 1992).

Statistical analyses

I conducted two different sets of analyses using generalized linear models (GLMs) and repeated measures analyses of covariance (RM ANCOVA): I tested (i) the effects of neighbour colony presence (denoted "1") or absence (i.e. colonies grown in isolation; denoted "0"), and (ii) the effects of neighbour MI-MR and focal MI-MR on focal colony survival at 20 weeks (c.f. Pettersen et al. 2016, Pettersen et al. 2020), growth, the *per capita* reproductive output over time, and the cumulative reproductive output after 24 weeks (i.e. an individual's summed reproductive output across the life-history), respectively. For survival, I conducted a binomial GLM with a logit-function, with focal MI-MR (continuous fixed effect), panel (categorical fixed effect), and either neighbour colony presence/absence (categorical fixed effect; "1" or "0") or neighbour MI-MR (continuous fixed effect) included in the model. For the cumulative reproductive output, I conducted a quasi-Poisson GLM with a log-link function using the same model structure as above. For growth and the *per capita* reproductive output over time, I conducted RM ANCOVAs with focal MI-MR (continuous fixed effect), panel (categorical fixed effect) and time (measurement points; categorical fixed effect), and either neighbour colony presence/absence (categorical fixed effect; denoted "1" or "0") or neighbour MI-MR (continuous fixed effect) included in the model. As the response variable, I used either size (number of zooids; $\log_{10}$-transformed prior to analyses) or the *per capita* reproductive output ($\log_{10}$-transformed prior to analyses) of focal colonies at each measurement point, respectively.

For all analyses, I first fit full models and reduced these where appropriate by removing non-significant interactions (assessed from log-likelihood ratio tests for binomial GLMs or *F*-ratio tests for Gaussian RM ANCOVAs and quasi-Poisson GLMs; where $\alpha > 0.05$). For focal colony survival, I found significant three-way interactions (panel × focal MI-MR × neighbour colony presence/absence and panel × focal MI-MR × neighbour MI-MR),

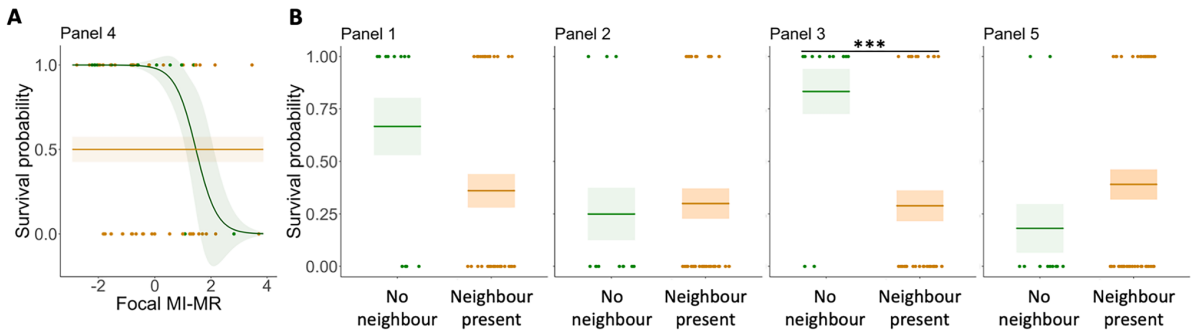

Figure 1: The effect of (A) neighbour colony presence/absence and focal MI-MR and (B) neighbour colony presence/absence on focal colony survival probability on each panel. Green and orange dots show underlying data points for focal colonies grown in the absence or presence of a neighbour colony, respectively; lines show predicted survival probability from generalized linear models (± 95% CI).

which were driven by one panel (see Results). I, therefore, performed additional analyses but excluded this panel to test for main effects and their interactions on focal colony survival on the other panels. I performed pairwise t-tests to compare survival and cumulative reproductive outputs between focal colonies that grew in the presence of a neighbour colony and focal colonies that grew in the absence of a neighbour colony on each panel. I conducted all statistical analyses in R version 3.6.2 (R Core Team 2017) using the packages *lmerTest* (Kuznetsova et al. 2017) and *car* (Fox and Weisberg 2019).

Results

The effect of neighbour colony presence/absence on focal colony performance

After 20 weeks in the field, I found that focal colony survival depended on the interaction between neighbour colony presence/absence and focal MI-MR, but the nature of this interaction varied in space (panel × neighbour colony presence/absence × focal MI-MR: χ^2 = 6.8, df = 1, P = 0.009). On one panel, focal colonies with a lower metabolic rate survived

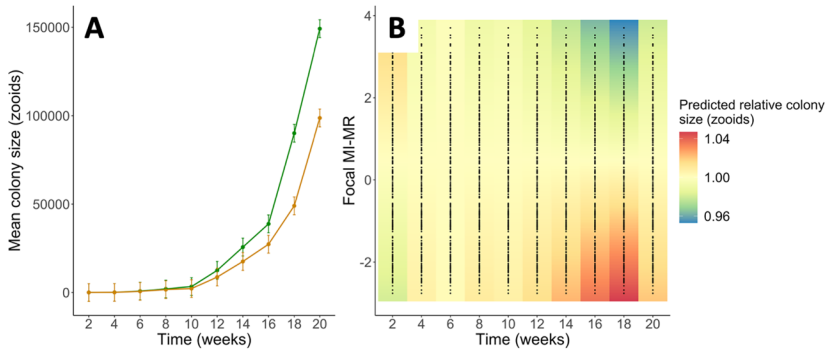

Figure 2: (A) Mean colony size (as number of zooids) of focal colonies grown in the absence (green line) or presence of a neighbour colony (orange line) plotted against time (in weeks). (B) The effect of focal MI-MR on relative colony size (as number of zooids; standardized to the mean) over time (in weeks). Error bars in (A) indicate standard errors. Black dots in (B) show underlying data points; warmer colours depict larger relative colony sizes.

better in the absence of a neighbour colony, but focal MI-MR did not affect survival if a neighbour colony was present (Fig. 1A; Panel 4). Focal MI-MR did not affect focal colony survival on the other four panels (Panels 1-3 and 5; panel × focal MI-MR: $\chi^2 = 2.69$, $df = 1$, $P = 0.1$; focal MI-MR: $\chi^2 = 0.84$, $df = 1$, $P = 0.36$; Fig. 1B). Instead, I detected a significant panel × neighbour colony presence/absence interaction effect on focal colony survival on these panels ($\chi^2 = 13.44$, $df = 1$, $P = 0.0002$), whereby neighbour colony presence decreased focal colony survival on one panel.

The presence of a neighbour colony invariably reduced focal colony growth, with focal colonies being on average 33.8% smaller in terms of zooid number after 20 weeks in the field compared to focal colonies grown in the absence of a neighbour colony (time × neighbour colony presence/absence: $F_{9,2011} = 2.49$, $P = 0.008$; Fig. 2A). Focal colonies with lower metabolic rates consistently grew more (time × focal MI-MR: $F_{9,2011} = 2.89$, $P = 0.002$; Fig. 2B), regardless of whether a neighbour colony was present (time × neighbour colony

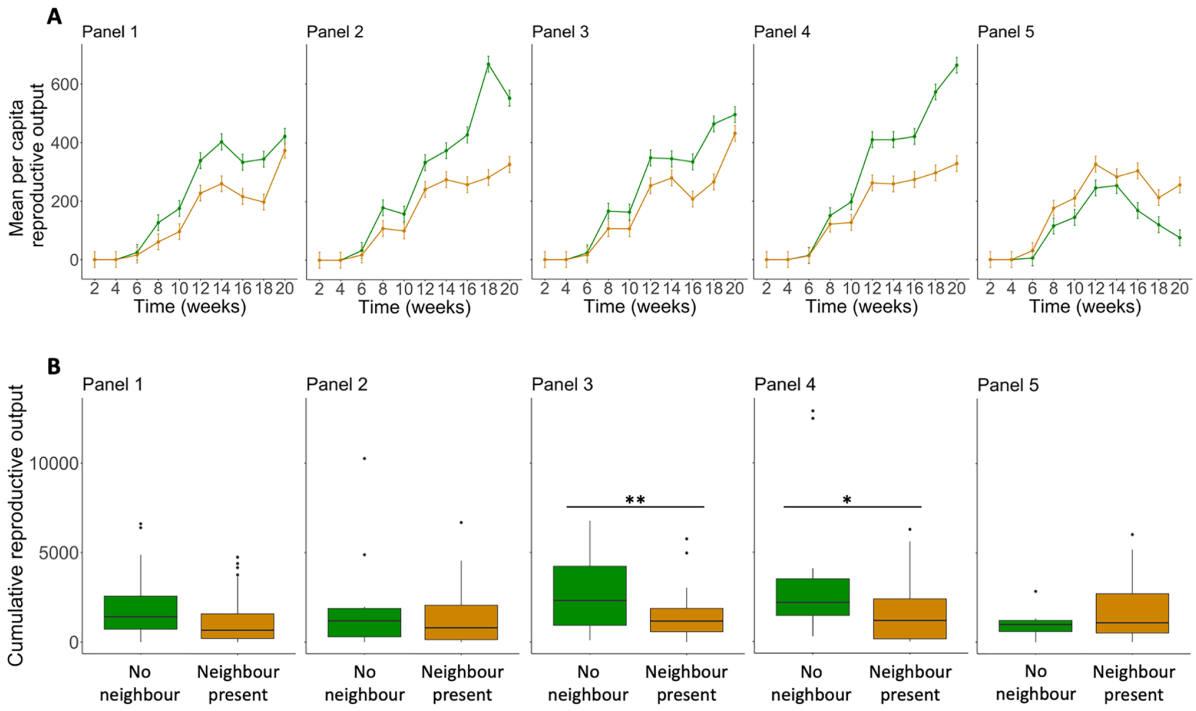

Figure 3: The effect of neighbour colony presence/absence on (A) the mean *per capita* reproductive output of focal colonies over time (± 1 SE); and (B) the cumulative reproductive outputs of focal colonies on each panel. Lines in (A) show mean *per capita* reproductive outputs of colonies grown in the absence (green) or presence of a neighbour colony (orange). Boxplots in (B) show the distribution of cumulative reproductive outputs for colonies grown in isolation (green) or in the presence of a neighbour colony (orange).

presence/absence × focal MI- MR: $F_{9,2011} = 0.65$, $P = 0.75$).

In terms of *per capita* reproductive output, I found that the effect of neighbour colony presence/absence differed across panels and over time (panel × neighbour colony presence/absence × time: $F_{36,1975} = 1.42$, $P = 0.05$). The interaction was driven by one panel, on which focal colonies had relatively higher *per capita* reproductive outputs if a neighbour colony was present (Fig. 3A; Panel 5). On the other four panels, colonies grown without a neighbour colony had overall higher *per capita* reproductive outputs (Fig. 3A; Panels 1–4). Focal MI-MR did not affect *per capita* reproductive outputs of focal colonies (focal MIMR ×

Table 1: Summary of survival, growth and reproductive outputs, and the various effects of neighbour colony presence/absence across all experimental panels. Purple indicates the response variable increased with neighbour colony presence, red indicates the response variable decreased with colony presence and with higher focal metabolic rate. The significance levels of neighbour colony presence/absence effects within each panel are presented in Table S1.

Performance metric	**Panel 1**	**Panel 2**	**Panel 3**	**Panel 4**	**Panel 5**
Survival					
Neighbour colony presence					
Neighbour colony presence × focal MI-MR					
Growth					
Neighbour colony presence					
Time × focal MI-MR					
Time × neighbour colony presence					
***Per capita* reproductive outputs**					
Time × neighbour colony presence					
Cumulative reproductive outputs					
Neighbour colony presence					

time: $F_{9,1975} = 0.43$, $P = 0.92$), regardless of whether another colony was present or absent (focal MI-MR × neighbour colony presence/absence × time: $F_{9,1975} = 1.45$, $P = 0.16$).

In terms of cumulative reproductive outputs of focal colonies (i.e. the summed reproductive outputs across each census date), the effect of neighbour colony presence/absence varied in space ($F_{4,254} = 2.25$, $P = 0.06$). On two panels, colonies grown in the absence of a neighbour colony produced on average 46% more offspring than colonies that were grown in the presence of a neighbour colony (Fig. 3B). Focal MI-MR did not affect

cumulative reproductive outputs of focal colonies ($F_{1,254}$ = 2.54, P = 0.11), although focal colonies with lower metabolic rates tended to have higher reproductive outputs.

My results pertaining to the effects of neighbour colony presence/absence are summarised in Table 1.

The effect of neighbour MI-MR on focal colony performance

Survival of focal colonies depended on the metabolic rate of both the focal colony and the neighbouring colony, and these effects varied in space (panel × focal MI-MR × neighbour MI-MR: χ^2 = 5.33, df = 1, P = 0.02). On one panel, I found that focal colonies with a lower metabolic rate survived better if they were paired with a low metabolic rate neighbour colony (Fig. 4A; Panel 4). On the other panels, focal colony MI-MR did not affect focal colony survival (Panels 1-3 and 5; χ^2 = 1.05, df = 1, P = 0.3). However, neighbour metabolic rate affected focal colony survival on some panels but not on others (χ^2 = 7.29, df = 1, P = 0.007). On two panels, I found a positive relationship between neighbour MI-MR and focal colony survival (Fig. 4B; Panels 1 and 2), but on the other two panels there was no effect of neighbour MI-MR (Panels 3 and 5; χ^2 = 0.23, df = 1, P = 0.63).

I detected a significant effect of neighbour MI-MR on the reproductive output of focal colonies, but the effect differed across panels and (panel × neighbour MI-MR × time: $F_{36,1507}$ = 1.53, P = 0.02). Overall, the neighbour MI-MR effect was strongest when reproduction began (Fig. 5A) – focal colonies paired with a low metabolic rate neighbour colony had relatively higher reproductive outputs on all except one panel (Panel 1), where the effect was reversed. After eight weeks, the effect of neighbour MI-MR on focal colony *per capita* reproductive outputs persisted over time on two panels (Panels 1 and 4) but dissipated on two other panels (Panels 3 and 5). On one panel (Panel 2), focal colonies paired with a low

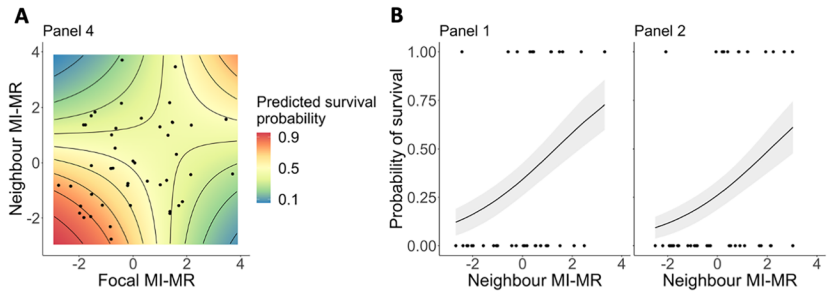

Figure 4: The effect of (A) focal MI-MR and neighbour MI-MR and (B) neighbour MI-MR on focal colony survival probability on each panel. Black dots show underlying data points. (A) Warmer colours depict a higher predicted survival probability. Lines in (B) show predicted survival probability from generalized linear models (± 95% CI).

metabolic rate neighbour colony reproduced more during early stages, but the effect changed in sign at 10 weeks and focal colonies paired with a high metabolic rate neighbour colony reproduced more thereafter. Focal MI-MR did not affect *per capita* reproductive outputs of focal colonies (focal MI-MR × time: $F_{9,\ 1507} = 0.82$, $P = 0.59$).

Neighbour MI-MR also affected the cumulative reproductive output of focal colonies, but, again, the effect differed across panels ($F_{4,195} = 2.9$, $P = 0.02$). Here, on two panels, I found either a positive (Panel 1) or negative relationship (Panel 4) between neighbour MI-MR and cumulative reproductive outputs of focal colonies (Fig. 5B). On the other three panels, I could not detect an effect of neighbour MI-MR ($F_{1,119} = 0.24$, $P = 0.62$) on the cumulative reproductive output of focal colonies (Fig. 5B; Panels 2, 3 and 5). These effects on cumulative reproductive outputs mostly reflect my results for weekly reproductive rates – when effects persisted through time, they were reflected in cumulative reproductive outputs. Focal MI-MR, in turn, did not affect cumulative reproductive outputs of focal colonies ($F_{4,195} = 0.96$, $P = 0.33$).

My results pertaining to the effects of neighbour metabolic rate are summarised in Table 2.

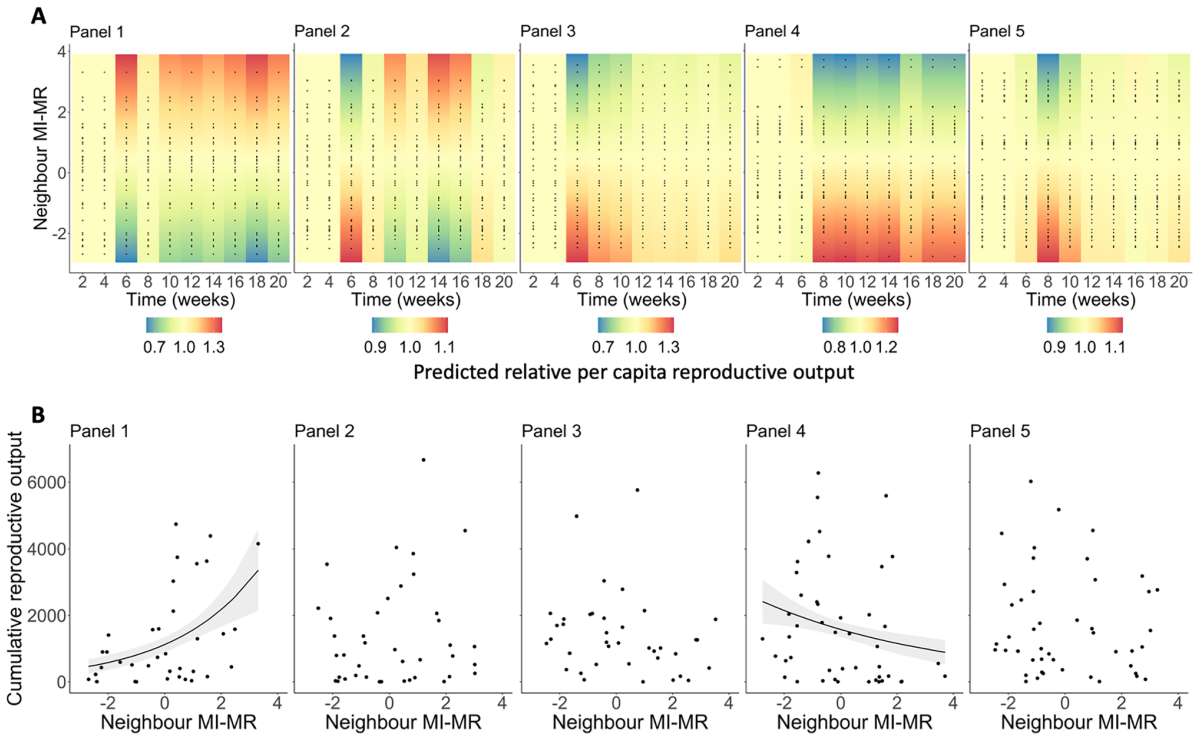

Figure 5: The effect of neighbour MI-MR on (A) the relative *per capita* reproductive output (standardized to the mean) over time (in weeks); and (B) the cumulative reproductive outputs of focal colonies on each panel. Black dots in (A) and (B) show underlying data points. Warmer colours in (A) depict higher relative *per capita* reproductive outputs. Lines in (B) are the predicted lines of best fit from generalized linear models (± 95% CI).

Discussion

I found that the metabolic phenotype of conspecific neighbour colonies altered the performance of focal colonies, but these effects varied in space. On most panels, I observed competitive effects – neighbouring colonies reduced the performance of focal colonies, and these effect were worsened when those neighbour colonies had higher metabolic rates. The effects of metabolic phenotype (of both the focal individuals and its neighbour) were complex and pervasive, affecting survival, growth and lifetime reproductive outputs, but the

Table 2: Summary of survival, growth and reproductive outputs, and the various effects of neighbour metabolic rate across all experimental panels. Yellow indicates the response variable increased with neighbour metabolic rate, blue indicates the response variable decreased with neighbour metabolic rate. Green indicates an interaction between both neighbour and focal metabolic rate. Colour gradients indicate a change in sign of the effect over time; e.g., a change from a negative to a positive effect (blue to yellow) or a change from a negative to no effect (blue to white). I used simple main-effects tests to determine the significance of main effects (and their interactions) within each panel.

Performance metric	Panel 1	Panel 2	Panel 3	Panel 4	Panel 5
Survival					
Neighbour MI-MR					
Neighbour MI-MR × focal MI-MR					
***Per capita* reproductive outputs**					
Neighbour MI-MR					
Time × neighbour MI-MR					
Cumulative reproductive outputs					
Neighbour MI-MR					

strength and even the sign of these effects varied in space and potentially with local resource regimes. Overall, these findings suggest a strong context-dependence of metabolic rate effects on conspecific interactions of *B. neritina* colonies.

I found that individuals with higher metabolic rates grew less than individuals with lower metabolic rates. Recent studies have shown that individuals with lower metabolic rates often grow more and reach larger body sizes due to their relatively lower maintenance costs (Burton et al. 2011, Pettersen et al. 2018), particularly when *per capita* resource availabilities are low (Reid et al. 2011, Reid et al. 2012, Auer et al. 2015, Zeng et al. 2017a, Zeng et al. 2017b, Auer et al. 2020). Within a population where conspecifics compete for resources, a lower metabolic rate may therefore confer a growth advantage when resources are limiting.

Colonies growing in the presence of a neighbour with a lower metabolic rate and higher growth tended to grow and reproduce more themselves. This benefit may have arisen for one of two reasons: 1) neighbour colonies with lower metabolic rates fed less voraciously, leaving more food for the focal colonies (this seems unlikely given these neighbour colonies were larger overall, and so have higher total resource consumption rates); or 2) neighbour colonies with lower metabolic rates and therefore larger colony sizes may have altered local flow regimes to benefit focal colonies more. Previous studies in this system indicate that conspecific colony size is a key mediator of the delivery of resources to interacting individuals (Cameron et al. 2017, Cameron and Marshall 2019) and I suspect size, rather than *per capita* resource-consumption drives my results here. In aquatic systems (including my own), the physical structure of sessile organisms can disrupt boundary currents and increase resource entrainment, particularly when water currents are too fast (Cameron et al. 2019, Okamura 1984, Cameron and Marshall 2019, Svanfeldt et al. 2017). Thus, it is possible that focal colonies benefited from being adjacent to fast growing, low-metabolic rate neighbour colonies on panels where flow was higher as they baffled the current more. I also found that the effects of the metabolic rate of neighbour colonies differed in persistence and sign across my replicated panels – I suspect this variable effect arises because of small scale differences in currents. On high flow panels, focal colonies may have benefited from low-metabolic rate, large neighbour colonies baffling flows, whereas on low flow panels, focal colonies suffered in the presence of such baffling (Svanfeldt et al. 2017).

I only investigated pairwise interactions between conspecifics, but intraspecific interactions occur across a range of densities in nature. Population density has been shown to affect the mode of competition (Cameron et al. 2007) as well as mediate transitions between competition and facilitation among species at least (Cameron et al. 2019). Similarly, the frequency of a given metabolic phenotype within a population may alter the outcome of

interactions among conspecifics (Ayala and Campbell 1974). Therefore, an important next step would be to orthogonally manipulate both the density *and* frequency of individuals of known metabolic phenotypes within a population and test for facilitative and competitive interactions.

Among species, context-dependent changes in the strength of competitive interactions is an important maintainer of species coexistence (Chesson 2000a, b, Hart and Marshall 2013). Similarly, it would be reasonable to expect that spatial variability in strength and direction of conspecific interactions maintain within-population variation in metabolic rate (Pettersen et al. 2020). Although I found differences in conspecific interactions among microenvironments, the metabolic phenotype of focal colonies covaried with growth but had little effects on their survival or reproductive fitness. Specifically, I found that focal metabolic rate affected survival of focal colonies on one panel (interacting with the metabolic rate of the neighbouring colony), but I could not detect an effect of focal metabolic rate on either survival or reproductive outputs on the other panels. Instead, the performance of focal individuals on these panels was more consistently affected by the metabolic phenotype of their neighbour colony. Neighbour metabolic rate affected the survival, growth and reproduction of focal colonies, albeit in contrasting ways that varied in space. These results suggest that complex eco-evolutionary feedbacks (akin to Indirect Genetic Effects, sensu Wolf et al. 1998) are likely to maintain variation in metabolic rate, despite previous studies showing strong directional selection (that should erode phenotypic variation) on focal metabolic rate in this system (Pettersen et al. 2020).

That the metabolic rate of the neighbour colony had more consistent and stronger effects on focal colonies than did the metabolic rate of those colonies themselves is remarkable. Most studies to date have focused on the covariance between the focal organism's metabolic rate and the performance of that organism (Pettersen et al. 2018). I can

find few examples of studies that explore how the metabolic rate of one individual affects the performance of other individuals (Auer et al. 2020). Yet it is well understood that metabolic rate covaries with any number of traits that determine how an organism will interact with and affect its environment and other species (e.g. body size, foraging rate, resource use; Careau et al. 2008, Biro and Stamps 2010a, Cameron et al. 2019). Thus, in this context, perhaps my results are less surprising than they first appear.

I recommend that future studies of the ecological effects of metabolic rate expand their scope to include multiple species and where possible, be done under field conditions. I predict that variation in metabolic rate is likely to have effects that extend beyond the focal organism but for the most part, these effects are unexplored. My study highlights the importance replicating arrays of competitors under natural conditions. I replicated my response surface design in space and found very different effects from one panel to another – in the absence of such replication, I would have overestimated the consistency of metabolic effects and drawn potentially misleading conclusions about how metabolic rate affects competitive interactions. Instead, I found that, while the metabolic phenotypes of both focal individuals and their neighbours always matter, their effects can differ in strength and direction – capturing this variability is necessary for a complete understanding of such metabolic rate effects in nature. Future studies are necessary to determine why I see such variable effects of metabolic rate in space but I suspect small scale variation in current regimes and the delivery of resources (Svensson and Marshall 2015) – future studies should manipulate local food availability to determine its role.

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Supplementary material

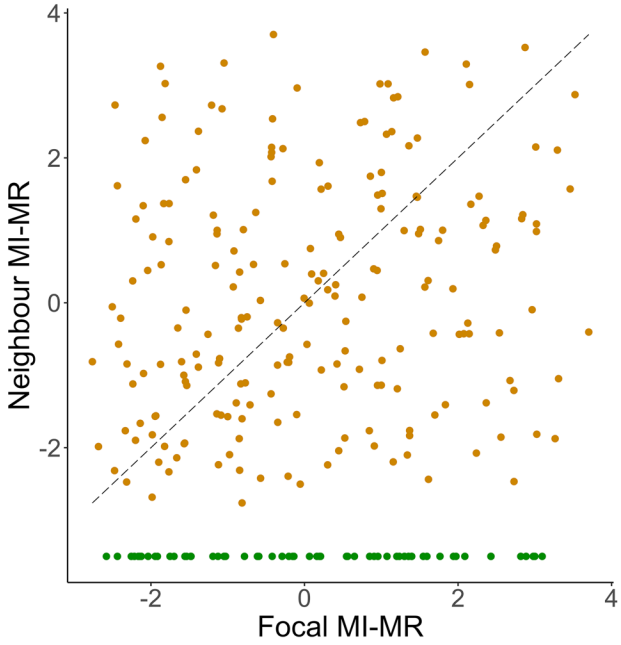

Figure S1: Schematic of the trait-specific, response-surface design used to test the effects of metabolism on pairwise interactions between *Bugula neritina* colonies. The orange points show the combinations of focal and neighbour mass-independent metabolic rates (MI-MR) used in pairwise interactions (n = 206), the green points show the mass-independent metabolic rates of colonies grown without a neighbour (n = 59). The grey-dashed line indicates equivalences between focal and neighbour MI-MRs.

Table S1: Outcome of pairwise t-tests comparing the survival or cumulative reproductive outputs between focal colonies that were grown in the presence and focal colonies that were grown in the absence of a neighbour colony on each panel.

	Neighbour absent		**Neighbour present**		
	Mean	**SE**	**Mean**	**SE**	***P***
Survival					
Panel 1	0.67	0.14	0.36	0.08	0.07
Panel 2	0.25	0.13	0.3	0.07	0.74
Panel 3	0.83	0.11	0.29	0.07	**0.0006**
Panel 5	0.18	0.12	0.39	0.07	0.2
Cumulative reproductive outputs					
Panel 1	2202.08	2363.78	1276.22	238.66	0.11
Panel 2	2009.08	842.51	1358.18	243.64	0.31
Panel 3	2795.67	667.78	1402.11	197.74	**0.009**
Panel 4	3744.75	1250.82	1615.39	254.73	**0.01**
Panel 5	1004.73	224.93	1696.83	225.91	0.15

Chapter 6

General discussion

Variation in metabolic rate both among and within populations has long intrigued physiologists. Although metabolic theory has proposed several hypotheses to explain the drivers and the maintenance of this variation in metabolic rate in time (Burton et al. 2011, Glazier 2005, Pettersen et al. 2018), its consequences for both individual- and population-level processes remain largely unknown. My work provides new insights into the drivers of variation in metabolic rate in a sessile marine invertebrate, *Bugula neritina*, as well as its consequences for individual performance and population-level dynamics in the field. In particular, I show that individuals change their metabolic rates in response to variable food availabilities (Chapter 2) and different environmental conditions (Chapter 3), but such plasticity in metabolic rate is not necessarily adaptive (Chapter 3). Furthermore, I show that metabolic rate can interact with other traits such as body size and traits that determine resource acquisition to affect individual performance (Chapter 3) as well as population-level demographic processes (Chapter 4) and intraspecific competition (Chapter 5).

Metabolic rate is plastic, but such plasticity is not always adaptive

In the field, phenotypic plasticity in metabolic rate in response to changing environmental conditions is widespread across taxa (reviewed in Norin and Metcalfe 2019). For example, individuals often change their metabolic rates in response to changes in temperature (Clarke 2017) or food availability (Auer et al. 2015, Naya et al. 2009, O'Connor 2000, Schimpf et al. 2012). Consistent with previous findings, my work in Chapters 2 and 3 shows that metabolic rate in *B. neritina* is strongly affected by prevailing feeding and environmental conditions. Specifically, I show that individuals increased their metabolic rates after feeding and decreased them during starvation (Chapter 2). Similarly, individuals had reduced metabolic

rates when grown in a harsh environment, where individuals were exposed to higher sedimentation rates and UV radiation compared to a benign environment (Chapter 3). Whether such metabolic plasticity is adaptive, however, was previously unclear, especially under field conditions. In Chapter 3, I provide one of the first formal tests of the adaptive significance of metabolic plasticity in the field by coupling estimates of metabolic plasticity in response to a shift from a benign to a harsh environment with formal estimates of phenotypic selection on metabolic rate under these environmental settings. Although individuals transplanted to the harsh environment expressed relatively lower metabolic rates to those that remained in the benign environment, a lower metabolic rate did not confer a fitness advantage in the harsh environment – suggesting that the observed metabolic plasticity was not adaptive.

When traits differ dramatically among environments, it is tempting to infer that such differences are driven by adaptive plasticity. Particularly since metabolic rate is tightly linked to an individual's resource demands (Brown et al. 2004, Burton et al. 2011), one might expect that adjusting the metabolic phenotype to prevailing environmental conditions would confer a fitness advantage. Many plastic phenotypes, however, are the consequences of a 'passive' response to environmental stress (van Kleunen and Fischer 2005) and may, therefore, not be adaptive or may even be maladaptive (Schlichting and Pigliucci 1995, Schmalhausen 1949, Smith-Gill 1983, Thompson 1991). Such passive responses may evolve due to genetic correlations with other traits that are under selection or due to genetic drift (van Kleunen and Fischer 2005). My findings therefore suggest that metabolic plasticity may merely represent a passive response due to correlations with other traits. Nevertheless, further field tests are needed in order to uncover whether metabolic plasticity may be adaptive in other species.

Metabolic rate likely interacts with other traits to drive individual- and population-level processes

Metabolic rate is genetically correlated with a range of traits including body size (White et al. 2019), growth rate (Sadowska et al. 2009), and exploratory and foraging behaviour (Biro and Stamps 2010, Careau et al. 2008). My work in Chapters 3, 4, and 5 shows that metabolic rate can interact with other traits such as body size and foraging behaviour in order to drive processes at both the individual and the population level. Below, I expand on the interactions I observed in this thesis.

Metabolic rate and body size

Metabolic rate and body size are strongly correlated (White et al. 2019). Yet, I show that, when accounting for differences in body size, mass-independent metabolic rate and body size can interact to affect individual performance (Chapter 3). Specifically, I found that both a low and a high metabolic rate can be advantageous (in terms of reproductive output) within a population, but this relationship depends on colony size. In Chapter 5, I show that metabolic rate can drive differences in body size, which, in turn, can affect the strength of intraspecific competition and, therefore, population-level processes in the field.

In both chapters (Chapters 3 and 5), the interplay between metabolic rate and body size and their effects on individual performance and competition likely depended on environmental conditions. In aquatic systems like *B. neritina*, the physical structure of sessile organisms can disrupt boundary currents and increase resource entrainment, particularly in high water flow environments (Cameron and Marshall 2019, Okamura 1984). Larger individuals are also more likely to overcome boundary layers and access different resource pools and, therefore, relatively more resources (Okamura 1984).

At the individual level (Chapter 3), I found that the combination of a larger body size and a higher mass-independent metabolic rate resulted in the highest performance. Individuals with higher metabolic rates are thought to have an overall higher sustained energy throughput, which may allow them to take up more resources from the environment and allocate more energy towards fitness (Burton et al. 2011). In contrast, smaller individuals performed better if they had relatively lower mass-independent metabolic rates since these individuals were more limited in their access to resources. Overall, these findings indicate that the benefits of a metabolic phenotype depend on other traits (e.g., body size), suggesting that metabolic rate is unlikely to evolve independently of other traits (Kozłowski et al. 2020, White et al. 2019).

In pairwise interactions (Chapter 5), I found that individuals with higher metabolic rates grew smaller likely due to a limited *per capita* access to resources within a population (Antonovics and Levin 1980, Violle et al. 2010). Here, focal colonies paired with a smaller (i.e. higher metabolic rate) neighbour performed better – smaller colonies usually have lower feeding rates compared to larger colonies, which may be particularly important in low flow environments where resources are limited. Conversely, in high flow environments, focal individuals may have benefited by growing in the presence of a larger (i.e. lower metabolic rate) conspecific where, due to the physical structure of larger colonies, focal individuals were less limited in their resource access due to resource amelioration by their neighbour (Okamura 1984). Taken together, these findings suggest that metabolic rate can drive variation in other traits such as body size, which, in turn, can affect resource competition and, therefore, population-level demographic processes in the field.

Metabolic rate and foraging activity

At the individual level, metabolic rate and foraging activity are tightly linked, with individuals with higher metabolic rates usually foraging more voraciously or effectively (Biro and Stamps 2010, Chappell et al. 2007, McNab 1980). In Chapter 4, I show that such intake theory at the individual level is likely to scale up to affect population-level demography in the field.

Current metabolic theory predicts that metabolic rate should determine a population's carrying capacity – because higher metabolic rates are associated with higher resource demands (Brown et al. 2004), a population's carrying capacity should decrease with increasing metabolic rate (Damuth 1981, 1987, Hatton et al. 2015, Perkins et al. 2019). Yet, I found that populations consisting of higher metabolic rate individuals had higher reproductive outputs during early stages, although the cumulative reproductive outputs (i.e. population yields) were not affected by population metabolic rate. Given that individuals with higher metabolic rates are thought to forage more voraciously or effectively, they may be able to extract more resources from their environment (Biro and Stamps 2010, Chappell et al. 2007, McNab 1980) and thereby increase their overall access to resources within a population. My findings, therefore, suggest that metabolic rate likely not only sets resource demands (Brown et al. 2004), but also affects resource supply into a population (Burton et al. 2011). Thus, similar to my findings above, these findings suggest that metabolic rate can interact with other traits such as foraging activity to determine population-level demographic processes in the field.

Conclusions and future directions

Metabolic rate varies extensively among populations and among individuals within a population (Burton et al. 2011, Pettersen et al. 2018). The consequences of this variation for

individual- and population-level processes, however, remain largely unknown. This thesis provides new insights into the drivers of variation in metabolic rate and its consequences for both individual performance and population-level processes in the sessile marine invertebrate, *Bugula neritina* in the field. First, I show that metabolic rate changes in response to variable food availabilities and environmental conditions, but such metabolic plasticity is not necessarily adaptive. Second, I show that metabolic rate is likely to interact with other traits such as body size and traits that determine resource acquisition (i.e. foraging activity) to affect both individual performance and population-level processes in the field. In light of my findings, I encourage further field tests in order to improve our understanding of how metabolic rate can affect individual performance and how such individual-level processes can scale up to affect dynamics at higher levels of organization.

In this thesis, I investigated the consequences of variation in metabolic rate for individuals and populations in isolation. In the field, however, individuals and populations are likely to interact with other species – such interspecific interactions are an important driver of species coexistence and diversity (Hutchinson 1961, Levin 1970). Therefore, going forward, I believe that an important next step should be to investigate the consequences of intraspecific variation in metabolic rate for processes at the community and ecosystem level. To date, theory around species interactions and their coexistence has traditionally relied on variation among species to explain the maintenance of diversity, although a large fraction of the trait variation in nature occurs within, not just between species (Hart et al. 2016, Messier et al. 2010, Violle et al. 2012). Metabolic rate is tightly linked to an individual's resource demands (Brown et al. 2004, Burton et al. 2011), and as my findings now show, can also affect resource supply due to correlations with other traits (see also Pettersen et al. 2020). As such, field manipulations of the density and frequency of metabolic phenotypes – both within and among species – represents an important next step to understand how resource use and

acquisition may shape key ecological principles within natural communities and ecosystems (Hart et al. 2016).

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