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# Plastic but not adaptive: habitat-driven differences in metabolic rate despite no differences in selection between habitats

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Metabolic plasticity in response to different environmental conditions is widespread across taxa. 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, then 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 there was no evidence for different selection regimes in the different environments, colonies expressed different metabolic phenotypes depending on the environment they experienced. Furthermore, there was no relationship between the degree of plasticity expressed by an individual and their subsequent fitness. In other words, we found evidence for phenotypic plasticity in metabolic rate, but there was no evidence that this plasticity was adaptive. In the absence of estimates of performance, changes in metabolic rate should not be assumed to be adaptive.

Keywords: fitness, lifetime reproductive output, metabolic plasticity, pace of life, phenotypic plasticity, phenotypic selection

## Introduction

Metabolic rate determines the rate at which organisms transform resources from the environment, use energy and live (Hulbert and Else 2000, Brown et al. 2004, Auer et al. 2018, 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



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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 (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). 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, Violle et al. 2010, Ghedini et al. 2017). 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 (Pigliucci 2001, DeWitt and Scheiner 2004, Ghalambor et al. 2007). In addition, selection needs to favour different phenotypes in the different environments, such that no metabolic phenotype is superior across all environments (Pigliucci 2001, DeWitt and Scheiner 2004, Ghalambor et al. 2007). 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. 2016a). 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. 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 by 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 (Robertson 1966, Price 1970, 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 (Lande and Arnold 1983, Blows and McGuigan 2015), and can be 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, we examined 1) phenotypic selection on metabolic rate in a benign and a harsh environment, and 2) 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. We 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, or a benign environment (when growing on horizontal surfaces) (Hart and Marshall 2013). When exposed to high levels of UV radiation, whole-animal metabolic rates are often reduced likely due to lower activity, as previously shown in tadpoles (Alton et al. 2012). Similarly, sedimentation can clog the feeding structures of *B. neritina*, resulting in reduced food intake, which, in turn, can drive lower metabolic rates (Schuster et al. 2019). *Bugula neritina* growing in the harsh environment have also been shown to experience more intense resource competition along with reduced growth rates and reproductive outputs (Hart and Marshall 2013), all of which have been associated with lower

metabolic rates (DeLong et al. 2014, Ghedini et al. 2017, Pettersen et al. 2018). 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, we highlight the tractability of this system to follow growth, survival and lifetime reproductive outputs in the field. We formally estimated parameters related to selection, including the opportunity for selection ($I$), and linear ($\beta$) and non-linear ($\gamma$) selection gradients in the different environments across two experimental runs. By estimating phenotypic selection parameters combined with estimates of phenotypic plasticity, we were able to investigate whether metabolic plasticity is adaptive in *B. neritina* in the field.

## Material and methods

### Study species, site and field deployment

The colonial bryozoan *Bugula neritina* 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.

We collected ~80 sexually mature *B. neritina* colonies from the Royal Melbourne Yacht Squadron in Port Phillip Bay, Victoria, Australia (37°51''43.1''S, 144°57'51.2''E) in March and April 2018. To obtain individuals for our experiments, we spawned colonies according to standard procedures (Schuster et al. 2019). Briefly, we kept colonies in the laboratory in field-collected seawater in aerated tanks in the dark. After 48 h, we 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, we rinsed unsettled larvae from the acetate sheets and kept settlers in tanks with unfiltered seawater. The next day, we 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). We 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, we suspended the panels 1 m below the water surface with settlers facing down at the Royal Brighton Yacht Club (37°54'29.0''S, 144°58'51.9''E).

### Mass-independent metabolic rate

We 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). We calculated mass-independent metabolic rate (MI-MR) by regressing metabolic rate on colony mass (nonlinear regression of the form $MR = a \times M^b$, 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, we returned acetate sheets bearing settlers to the laboratory after colonies within each experimental run had been in the field for three weeks. We kept colonies in aerated tanks with field-collected seawater at 19°C overnight. Prior to metabolic rate measurements, we removed any epibionts and debris from the colonies. We 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. We measured metabolic rate using 5 ml (run 1) or 750 µl (run 2) glass vials at 19°C as described in Schuster et al. (2019). Although we measured metabolic rates in the laboratory rather than in the field, laboratory effects on individual metabolic rates (i.e. genotype by environment interactions; $G \times E$) were likely relatively small given the repeatability of metabolic rates observed generally (White et al. 2013, Auer et al. 2016b) and in *B. neritina* specifically.

To determine colony size of three weeks old colonies (hereafter original colony size), we counted the number of zooids in each colony – in our 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 we conducted measurements of colony size and metabolic rate.

Following metabolic rate measurements, we glued each colony onto a 25 $cm^2$ single acetate sheet, which we then assigned to a PVC plate (55 × 55 × 3 mm) with a unique ID number. We 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). We redeployed the panels back into the field but placed half of the panels (run1: n = 4; run 2: n = 6) 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, we attached colonies on vertically suspended panels at a similar depth to horizontally suspended panels (see the Supporting information for details on deployment). It is noteworthy that we 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

We 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. We measured the reproductive output of each colony as the cumulative number of ovicells throughout the duration of the experiment. In addition, we 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, we moved each plate to a different position within the assigned panel every two weeks (Mitchell-Olds and Shaw 1987, Rausher 1992). We randomised the position of each colony within their assigned panel only, we did not move colonies across panels. We 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

### *Testing for differences in reproductive outputs among environments and experimental runs*

To determine whether colony reproductive outputs differed among environments and experimental runs, we 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.

### *Estimating the opportunity for selection in the different environments*

We 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 $I = \sigma^2_W / \bar{W}^2$, where $\sigma^2_W$ is the variance in absolute fitness and $\bar{W}$ is the mean absolute fitness (Crow 1958). We calculated bootstrap confidence intervals using the R package *boot* ver. 1.3-24 (Davison and Hinkley 1997, Canty and Ripley 2019).

### *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, we 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.

### *Characterizing fecundity selection within and between environments*

To characterize selection within and differences in selection between environments, we 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. We 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. For most taxa, physiological traits such as metabolic rate are not invariant throughout an individual's life – metabolic rate changes over time (Auer et al. 2016a, White et al. 2013). Nevertheless, our estimates of selection on metabolic rates of three weeks old individuals are in line with standard studies of selection. Indeed, most studies focus on traits that are not fixed but are either restricted to a particular life-history stage (e.g. seed size, larval size; Marshall et al. 2018) or change over time (e.g. body mass, body condition, coloration; Kingsolver et al. 2001). For example, we know that initial size strongly affects fitness later in life, although these organisms do not have a constant size throughout their life-history (Marshall and Keough 2008, Marshall et al. 2018). In this sense, the effects of offspring size on fitness are indirect, but clearly, they matter. Analogously, the metabolic rate colonies have at three weeks old matters for subsequent performance. We also estimated selection on original colony size, and since colony sizes differed between experimental runs, we analysed each run separately due to non-overlapping covariance ranges. To estimate selection on MI-$MR_E$ and original colony size, we 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; our response variable) and our standardized traits of interest (our predictor variables: original colony size and MI-$MR_E$; each individual's trait value divided by the standard deviation of individuals' trait values within a panel) (Lande and Arnold 1983). We standardized fitness within each panel because colony reproductive outputs differed across panels. In run 2, six colonies did not reproduce, three of which did not survive to reproduce. We included these six colonies as '0' fitness in selection analyses

since the overall qualitative outcome did not change if we 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, we included panel ID as a categorical random effect, nested within environment (Mitchell-Olds and Shaw 1987). To begin with, we conducted an overall, formal test of whether selection differed between environments (benign versus harsh). We 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), we 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. We 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, we 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, we measured three key life-history traits.

#### *Growth*

We 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). We included run, environment and time (measurement points) as categorical fixed effects, and MI-$MR_E$ as the covariate of interest.

#### *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, we 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'. We then fit a logistic regression to the data, including run, environment, MI-$MR_E$ and their interaction as fixed effects.

#### *Longevity*

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

### Testing for phenotypic plasticity in metabolic rate

We were interested in how the environment (benign versus harsh) affected the colonies' metabolic rates. Hence, we 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). We determined MI-$MR_L$ as described above and redeployed colonies at the Royal Brighton Yacht Club 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. We then used a linear mixed effects model to test for the effects of MI-$MR_E$, environment, run and their interaction on MI-$MR_L$. We included MI-$MR_E$, environment and run as fixed effects.

### Environment-dependent covariance between an individual's degree of plasticity and its growth and reproductive fitness

We found that individuals placed into a harsh environment had lower MI-$MR_L$ on average. We therefore calculated the reaction norm for each individual to investigate whether individuals that changed their metabolic rates more or less had higher fitness (in terms of growth and reproductive outputs), and whether fitness effects associated with the degree of plasticity differed between environments. To derive reaction norms, we subtracted MI-$MR_E$ of each individual from its MI-$MR_L$ (i.e. MI-$MR_L$–MI-$MR_E$; hereafter ΔMI-MR). We then tested for effects of ΔMI-MR on growth (number of bifurcations over time during the first 25 weeks) by using a repeated measures ANCOVA with environment and time (measurement points) included as categorical fixed effects, and ΔMI-MR as the covariate of interest. To test for effects of ΔMI-MR on cumulative reproductive outputs, we used a linear mixed effects model with ΔMI-MR, environment and their interactions included as fixed effects. Given that the degree of plasticity and individuals' reaction norms differed between experimental runs, we analysed each run separately due to non-overlapping covariance ranges.

We conducted all analyses in R ver. 3.6.2 (<www.r-project.org>) using the packages *nlme* (Pinheiro et al. 2017) and *lme4* (Bates et al. 2007). In all models, we included panel ID as a categorical random effect, nested within environment. We compared the parsimony of models including fixed effects and their interactions to reduced models using the Akaike information criterion (AIC; Akaike 1973).

## Results

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

The effect of environment (benign versus harsh) on colony reproductive outputs differed between experimental runs

(Supporting information). 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 (Supporting information). Furthermore, the opportunity for selection (*I*) was higher in the harsh environment in both runs (Supporting information). *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).

## 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, we could not detect linear selection acting on either MI-$MR_E$ or original colony size, but we found support for significant non-linear selection (significant original colony size × MI-$MR_E$ interaction; Table 1, 2). When exploring the different forms of non-linear selection, we could not detect any quadratic selection, but we 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, we found support for positive linear selection acting on original colony size, with larger colonies having the highest fitness (Table 1, 2). We could not detect any linear selection acting on MI-$MR_E$ and we 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

### *Growth*

During the first 25 weeks, we 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, Supporting information). At 25 weeks, colonies in the harsh environment were on average 7.15% smaller than colonies in the benign environment.

### *Age at onset of reproduction*

The effect of environment on age at onset of reproduction depended on experimental run (Supporting information). In run 2, colonies in the benign environment developed ovicells earlier, on average after 10.08 (± 0.24 SE) weeks in the field,

Table 1. Linear mixed effects model for the relationship between total reproductive output and mass-independent metabolic rate early (MI-$MR_E$) and original colony size. We built the model by adding each term one by one and tested the model fit using the Akaike information criterion (AIC). In all analyses, we included panel as a random effect, nested within environment. Numbers in bold indicate terms that significantly improved the model fit. df = degrees of freedom, logLik = log-likelihood; AICc = corrected AIC.

| | df | logLik | AICc |
|---|---|---|---|
| Exp. run 1 | | | |
| Intercept | 3 | −1055.6 | 2117.4 |
| Environment | 4 | −1054.3 | 2117 |
| MI-$MR_E$ | 5 | −1053.9 | 2118.4 |
| Original colony size | 6 | −1052.6 | 2118 |
| Environment × MI-$MR_E$ | 7 | −1052.6 | 2120.2 |
| Environment × original colony size | 8 | −1051.1 | 2119.4 |
| MI-$MR_E^2$ | 9 | −1051.1 | 2121.8 |
| Original colony size$^2$ | 10 | −1050.3 | 2122.7 |
| Environment × MI-$MR_E^2$ | 11 | −1049.3 | 2123 |
| Environment × original colony size$^2$ | 12 | −1049.1 | 2125.1 |
| MI-$MR_E$ × original colony size | 13 | **−1046.4** | **2122.2** |
| Environment × MI-$MR_E$ × original colony size | 14 | −1045.9 | 2123.9[†] |
| [†]$R^2 = 0.25$ | | | |
| Exp. run 2 | | | |
| Intercept | 3 | −1626.7 | 3259.6 |
| Environment | 4 | **−1618.6** | **3245.5** |
| MI-$MR_E$ | 5 | −1618.5 | 3247.4 |
| Original colony size | 6 | **−1608. 4** | **3229.2** |
| Environment × MI-$MR_E$ | 7 | −1607.5 | 3229.6 |
| Environment × original colony size | 8 | −1607.5 | 3231.8 |
| MI-$MR_E^2$ | 9 | −1606.9 | 3232.8 |
| Original colony size$^2$ | 10 | −1606.4 | 3234.1 |
| Environment × MI-$MR_E^2$ | 11 | −1606.4 | 3236.4 |
| Environment × original colony size$^2$ | 12 | −1606.2 | 3238.4 |
| MI-$MR_E$ × original colony size | 13 | −1606.1 | 3240.5 |
| Environment × MI-$MR_E$ × original colony size | 14 | −1605.5 | 3241.5[††] |
| [††]$R^2 = 0.31$ | | | |

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. Values in bold indicate terms that significantly improved the model fit based on corrected AIC (AICc) estimates (Table 1).

| | | γ′ (± SE) | |
|---|---|---|---|
| | β′ (± SE) | Original colony size | MI-$MR_E$ |
| Exp. run 1 | | | |
| 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) |

whereas colonies in the harsh 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 (Supporting information).

### *Longevity*

Low metabolic rate colonies lived longer than higher metabolic rate colonies in both runs (Fig. 3), and the effect did not differ between environments (Supporting information).

### 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 (Supporting information). 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-$MR_L$, and the effect was stronger in run 1 (Supporting information). Here, individuals with initially higher metabolic rates also had higher metabolic rates later on, and vice versa.

### Environment-dependent covariance between ΔMI-MR and fitness

An individual's degree of metabolic plasticity (ΔMI-MR) did not affect its cumulative reproductive output in either run, and the effect was independent of the environment colonies were in (no significant environment × ΔMI-MR interaction; Supporting information). Thus, individuals that reduced their metabolic rates more or less did not reproduce more. Similarly, we could not detect an effect of ΔMI-MR on growth (Supporting information) in either environment (no

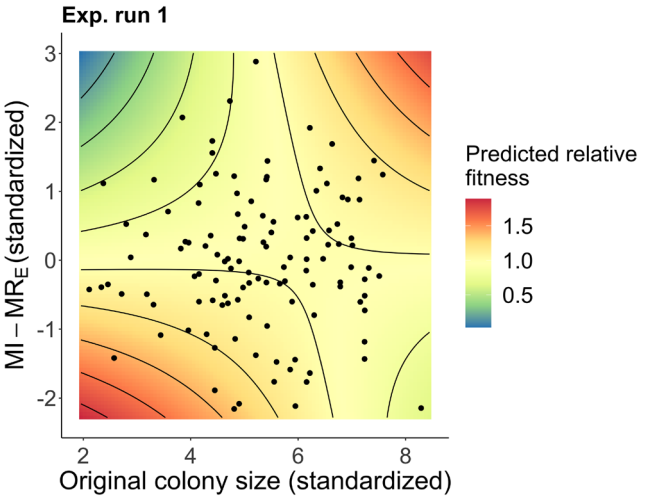


Figure 1. Predicted relative fitness (total lifetime reproductive output) plotted against mass-independent metabolic rate early (MI-$MR_E$) and original colony size for *B. neritina* colonies in experimental run 1 (n = 121). Black dots represent the underlying data points. Warmer colours indicate higher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

significant ΔMI-MR × environment × time interaction in either experimental run; Supporting information).

## Discussion

We 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. Our 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 we observed, it would have been tempting to infer that such a response increases fitness, but our comprehensive mapping of metabolic phenotype to fitness across environments contradicts this intuition. Our results are not a product of a lack of statistical power – earlier studies in our 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.

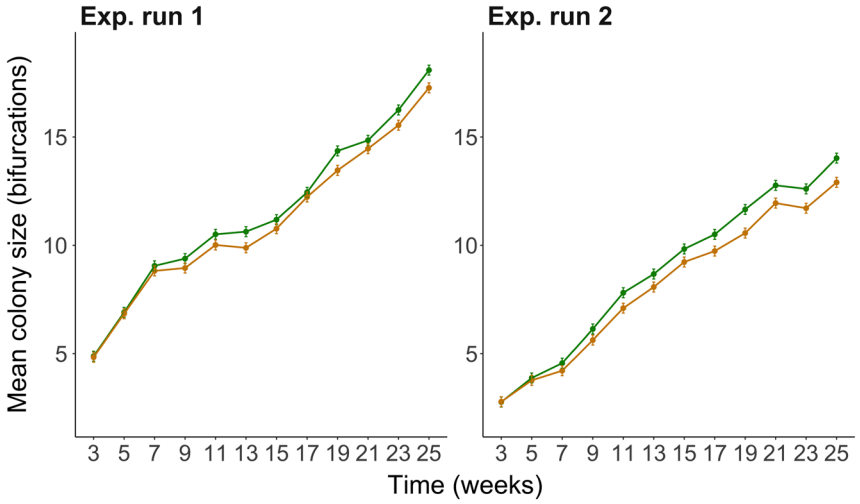


Figure 2. Mean colony size (as mean number of bifurcations) in the benign (green lines) and the harsh (orange lines) environment plotted against time (in weeks). Error bars show standard errors.

We found that colonies in the harsh environment had much reduced metabolic rates, but a lower metabolic rate was not advantageous in that environment. Similarly, individuals that reduced their metabolic rates more in response to the harsh environment did not have higher performance than individuals that were less plastic. We also could not detect any differences in selection between environments overall. Yet it is unlikely that we were unable to detect any environmental variation in selection due to low statistical power, since we 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). Similarly, there may be limits to physiological plasticity in response to environmental change that are at least partly set by biochemical constraints, resulting in fitness costs rather than benefits (Seebacher et al. 2015). Hence, metabolic plasticity may merely represent a passive response due to correlations with other traits or there may be limits to physiological plasticity due to biochemical constraints, 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.

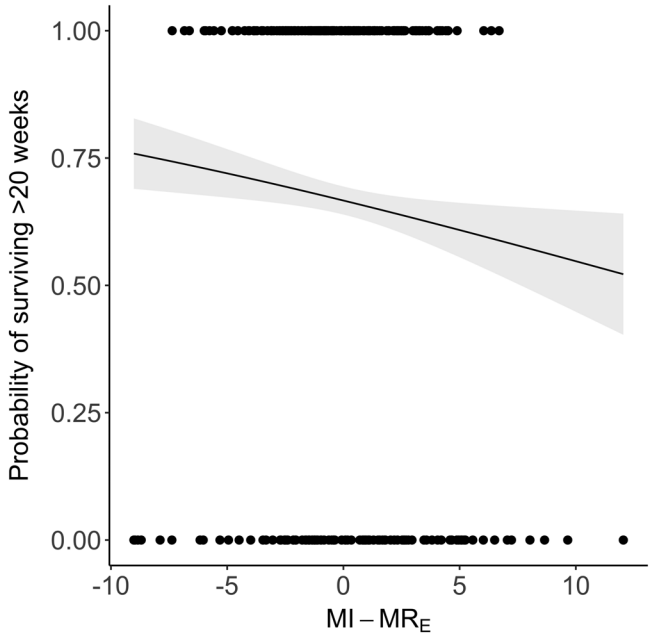


Figure 3. Logistic regression for predicted longevity (probability of surviving > 20 weeks) plotted against mass-independent metabolic rate early (MI-MR$_E$) for both experimental runs. Data points represent predicted MI-MR$_E$ for each colony.

Metabolic rate and body size are strongly correlated (White et al. 2019). Yet we 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, we 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 our 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). We 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

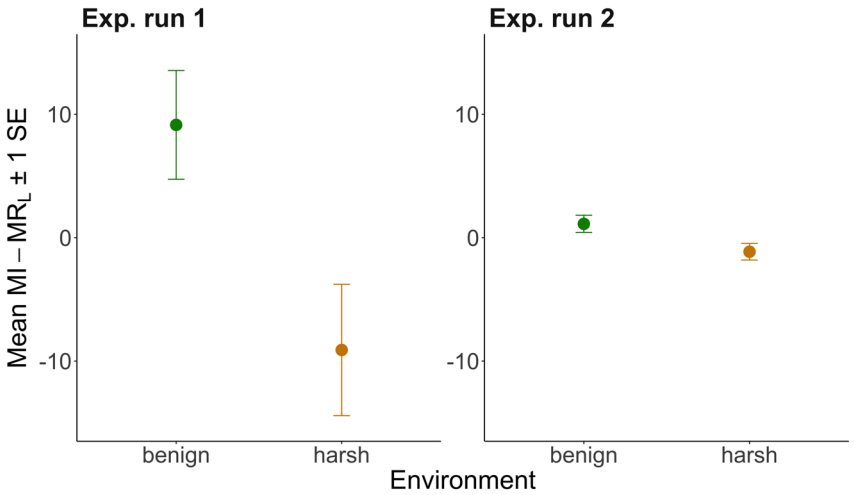


Figure 4. Mean mass-independent metabolic rate late (MI-MR$_L$ ± 1 SE; at 5 weeks) in the benign (green) and harsh (orange) environment, respectively, in each experimental run.

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 by Pettersen et al. 2018) – so why did we 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, we 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 our species (Pettersen et al. 2016, 2020), whereas we 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 (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, 2020) measured metabolic rates during the larval stage, whereas we 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. 2016b). Thus, the metabolic rates we measured were likely a product of small-scale environmental differences rather than the underlying metabolic phenotype of the organisms. Our findings, therefore, pertain to the metabolic phenotypes of three weeks old individuals as our 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.

We measured metabolic rates in the laboratory rather than under field conditions. Nevertheless, laboratory effects on individual metabolic rates were likely relatively small given the repeatability of metabolic rates generally (White et al. 2013, Auer et al. 2016b) and in *Bugula neritina* specifically – we found strong effects of MI-MR$_E$ on MI-MR$_L$, indicating some degree of repeatability of metabolic rates in this species. More importantly, we found strong environment effects on MI-MR$_L$, with individuals kept in the harsh environment having an overall lower MI-MR$_L$ than individuals kept in the benign environment. These findings suggest that a laboratory measured metabolic rate is a reliable proxy for an individual's field metabolic rate, given that individuals are kept in the laboratory for a short time only.

How may environmental heterogeneity alter the process and outcome of selection on metabolic rate? We 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, we could not detect any differences in the intensity or form of selection acting on metabolic rate in the different environments. Therefore, our findings suggest that although environmental heterogeneity has the capacity to alter variation in fitness, it may not affect the distribution of metabolic phenotypes in our 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 our 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 we 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.

### Data availability

Data are available from the Dryad Digital Repository: <http://doi.org/10.5061/dryad.p8cz8w9q3> (Schuster et al. 2021).

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### Author contributions

**Lukas Schuster**: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (equal); Project administration (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (equal). **Craig R. White**: Conceptualization (equal); Funding acquisition (supporting); Methodology (equal); Resources (supporting); Supervision (supporting); Validation (equal). **Dustin J. Marshall**: Conceptualization (equal); Funding acquisition (lead); Methodology (equal); Project administration (equal); Resources (equal); Supervision (lead); Validation (equal); Writing – review and editing (equal).

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