

# Selection on offspring size among environments: the roles of environmental quality and variability

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## Summary

1. How mothers balance the trade-off between offspring size and number to maximize maternal fitness has long been of interest to ecologists seeking to understand the evolution of offspring size. Predictions of the optimal offspring size depend fundamentally on the relationship between offspring size and offspring performance, which may in turn vary with environmental conditions.

2. Selection for larger offspring is expected to intensify as environmental quality deteriorates. Models also predict that variable selection on offspring size may favour the evolution of larger offspring than those favoured when selection is constant, or of strategies of variable offspring provisioning (e.g. bet-hedging, plasticity). To date, there is mixed empirical support for the first expectation and few tests of the second. Given, however, that offspring size effects are often estimated under controlled laboratory conditions that presumably downplay their strength and variability, we may not yet understand how selection shapes offspring size in nature.

3. We examined several relationships between offspring size and performance in controlled (laboratory) and natural (field) environments over time for a colonial marine invertebrate, *Bugula neritina*, and assessed the variability of these relationships by doing so for replicate cohorts. We further developed a simple optimality model to examine whether predictions of the optimal offspring size were similar (or similarly variable) across environments.

4. We found that selection on offspring size varied substantially among laboratory and field environments, and among cohorts in the latter. In the laboratory, our model consistently predicted that mothers should maximize their fecundity by producing the smallest possible offspring. In the field, however, the predicted optimal offspring size varied from the smallest possible size to the largest possible size for different cohorts.

5. Our study suggests that laboratory estimates of offspring size effects, though often necessary, may not always reflect the direction or variability of selection on offspring size under natural conditions. The optimal offspring size for mothers in nature may be an ever-shifting target that shapes provisioning strategies such as bet-hedging or plasticity in offspring size.

**Key-words:** egg size, maternal effects

## Introduction

Mothers must invest finite resources for reproduction in either a few, well-provisioned offspring or in more numerous offspring, each with less maternal provisioning (Stearns 1992). Understanding how mothers balance this trade-off between offspring size and number to maximize maternal fitness has been a goal of life history theory for over 60 years (Lack 1947; Bagenal 1969). In theory, the optimal offspring size (OOS) that mothers should produce depends

on the relationship between offspring size and offspring fitness (Smith & Fretwell 1974; Parker & Begon 1986; McGinley, Temme & Geber 1987). Models predict that if the relationship between offspring size and offspring fitness is strongly positive, mothers should increase their per-offspring investment because the fitness benefits of doing so outweigh the concomitant loss of fecundity. If the same relationship is weak, mothers should produce smaller offspring because the gain in fecundity exceeds the small fitness cost to individual offspring. Hence, the relationship between offspring size and offspring fitness (or some measure of performance that correlates with fitness) has long

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been of key interest to ecologists interested in the evolution of offspring size.

Theoretical models also predict that selection for larger offspring should be weaker (i.e. the slope of the relationship between offspring size and performance shallower) in more benign or high-quality environments (Parker & Begon 1986; Sibly & Calow 1986). Despite some empirical support for this prediction (e.g. Einum & Fleming 1999; Fox 2000), other empirical studies suggest that the role of environmental stress in mediating selection on offspring size is less straightforward. Allen, Buckley & Marshall (2008), for example, demonstrated selection for larger offspring under low levels of competition, but for smaller offspring under high levels of competition, whereas Moran & Emlen (2001) found selection for larger offspring size to strengthen, not weaken, with decreasing environmental stress. Hence, the generality of this prediction is currently unclear, not least due to the paucity of such tests. Notably, most studies that have failed to detect a relationship between offspring size and performance (see reviews by Bernardo 1996; Fox & Czesak 2000; and Marshall & Keough 2008b) have been conducted in carefully controlled laboratories or greenhouses that may reflect the types of benign conditions (e.g. with *ad libitum* food and/or few stresses on reproduction or survival; Boggs 2009) likely to minimize selection on offspring size.

There is increasing recognition, moreover, that estimates of the mean OOS are not in themselves sufficient to predict how offspring size may evolve: estimates of variation in such optima are also crucial. This is because unpredictable variation in the OOS may favour strategies of maternal investment that differ to those favoured when the optimal size remains constant. Simulation modelling by Einum & Fleming (2004), for example, shows that environmental stochasticity may select for larger offspring sizes than those predicted by traditional optimality models (e.g. Smith & Fretwell 1974). Such production of large, high-quality offspring, which may allow mothers to buffer their offspring's fitness (and hence their own) against environmental stochasticity, is known as conservative bet-hedging. Alternatively, the inability of mothers to anticipate the conditions faced by offspring (and hence their optimal size) in stochastic environments may select for the production of variable-sized offspring (known as diversified bet-hedging; e.g. Marshall, Bonduriansky & Bussiere 2008). That the OOS may depend not only on the relationship between offspring size and performance, but also on variability in this relationship, raises a further question about our current understanding of offspring size effects. We must clarify both the role of the environment in determining selection on offspring size and, perhaps more importantly, that of environmental variability in mediating such selection.

One way to address this issue is to examine the relationship between offspring size and offspring performance (and thus, in predictions of OOS) in controlled (laboratory) and natural (field) environments simultaneously. Of the few studies to do so, Einum & Fleming (1999) found the relationship between offspring size and performance in the brown trout, *Salmo trutta*, to be weaker and more transient in a cohort of individuals

reared in a controlled hatchery environment than for the same cohort (i.e. full siblings) reared under less favourable, semi-natural conditions. Similarly, Fox (2000) found the intensity of selection on offspring size, estimated for distinct field and laboratory populations of a seed beetle (*Stator limbatus*) on seeds of the same trees, to be ~30% greater and significantly more variable in the field. Although Einum & Fleming (1999) did not seek to formally compare variation in offspring size effects between controlled and natural environments (we suspect them to be inherently more variable in the latter than the former), nor Fox (2000) to explore genetic influences (within or among populations) on selection across such environments, their results jointly highlight the need to do so. If estimating the relationship between offspring size and performance in benign and/or carefully controlled environments tends to downplay not only its strength, but also its variability, we may not yet appreciate the prevalence of selection for bet-hedging strategies of offspring provisioning.

Here, we compare the relationship between offspring size and post-metamorphic performance across controlled (both stressful and more benign) and natural environments in a marine bryozoan, *Bugula neritina* (Linnaeus, 1758; Fig. 1), and assess the variability of this relationship in each environment by doing so for replicate cohorts. This species offers an excellent opportunity to conduct such a study because its sessile nature allows post-metamorphic performance to be measured under realistic field conditions, and because such performance is known to be strongly affected by offspring size (Marshall & Keough 2008b). Within each cohort, we followed the performance of individuals of known offspring size through early post-metamorphic life to adulthood in the laboratory and the field. Two measures of offspring performance – the time to starvation in the absence of food, and growth and/or survival given food *ad libitum* – are commonly



Fig. 1. Part of a *Bugula neritina* colony, showing individual zooids bearing ovicells (a) and with lophophores (b) extended to feed.

assessed in laboratory studies of offspring size effects (see reviews by Fox & Czesak 2000; and Marshall & Keough 2008b). The first is generally designed to test whether larger offspring (with presumably more resources than smaller offspring) can better withstand environmental stress in the form of food limitation, whereas the second explores whether larger offspring have an inherent advantage over smaller offspring under relatively benign conditions. To incorporate both measures into our study, we compared the effect of offspring size on the time to starvation among replicate cohorts exposed to food stress in the laboratory; for these same cohorts, we then compared offspring size effects on growth and survival between a benign laboratory environment (with food *ad libitum*) and a potentially harsher and/or naturally varying field environment. We then used our data to parameterize a simple optimality model to generate predictions of OOS under each set of conditions for each successive cohort.

## Materials and methods

### STUDY SPECIES AND SITE

*Bugula neritina* is an arborescent bryozoan that filter-feeds and grows via the asexual budding of zooids (Fig. 1). When colonies reach sexual maturity (3–8 weeks post-settlement), eggs are fertilized and brooded for approximately a week in conspicuous external chambers (ovicells) borne on individual zooids (Fig. 1; Woollacott & Zimmer 1975). Non-feeding larvae are usually released from ovicells at dawn (spawning is also induced by strong artificial light), and swim only briefly (minutes to hours) in the plankton before settling and metamorphosing onto suitable substrate (Marshall & Keough 2003). We collected sexually mature colonies from pier pilings at Scarborough Marina, Brisbane, Australia (27°10'8"S; 153°6'3"E), a site sheltered from strong winds and wave energy where *B. neritina* is abundant for much of the year.

### LARVAL MEASUREMENT AND SETTLEMENT

To minimize microenvironmental variation among replicate cohorts, all maternal colonies were sampled from a similar depth (*c.* 1 m below the water surface) and within a 100-m radius. Colonies were transported from the field to the laboratory, where they were held for 60 h at a constant temperature of *c.* 19–20 °C in light-proof aquaria of aerated seawater from the field site, before exposure to bright light to stimulate the release of larvae (these were pooled across 20 colonies in each replicate cohort). To measure larval size, each focal larva was isolated on a glass slide in a drop of seawater from the field site and photographed at 80× magnification using a digital camera mounted on a microscope. Cross-sectional area (a good predictor of larval volume; Marshall, Bolton & Keough 2003) was estimated to the nearest micron from images captured when the larva was oriented with either its ciliated groove or eyespots facing directly towards the camera. Throughout our study, we assume that larger offspring are energetically more costly to produce than smaller offspring (as argued by Marshall & Keough 2008b). Although it is unclear whether the energetic content of offspring scales directly with size (Moran & McAlister 2009), the precise nature of this relationship in *B. neritina* has no qualitative effect on our results. Digital images were analysed using Image-Pro Plus 5.1 (Media Cybernetics, Bethesda, Maryland, USA). Each larva was then placed alone in a petri dish (90 mm diameter) of seawater from the field

site. To encourage settlement and replicate field conditions, dishes were pre-roughened and coated with natural biofilms that developed over several days' immersion at the field site. Once settled, each focal settler was circled with pencil to distinguish it from others (of the same or different species) recruiting naturally to its dish after deployment. Settlers were given 24 h to metamorphose at a constant temperature of 22 °C before being allocated haphazardly to experimental treatments.

### EXPERIMENTAL DESIGN

We conducted the experiment on three replicate cohorts, each comprising 60 settled individuals of known larval size. Within cohorts, 20 settlers were allocated randomly to each of three experimental environments: (i) laboratory 'starved', (ii) laboratory 'fed' and (iii) the field site of parent colonies. The experiment was initiated on replicate cohorts in successive weeks of June 2008.

Settlers allocated to the 'starved' treatment were maintained at *c.* 19–20 °C (similar to field conditions at this time) in plastic aquaria containing 10 L of field-collected seawater filter-sterilized to 0.22 µm to remove the most potential food sources while maintaining natural salinity and chemistry. Each cohort in this treatment was contained in a single aquarium whose field-collected water was completely replaced every third day. To keep attached settlers suspended in a similar orientation to their natural habit, their dishes were threaded onto stainless-steel rods propped within aquaria (dishes were shuffled between rods at each water change). Bubbler were placed in all aquaria to maintain aeration and gentle water movement. As field colonies grew in full shade, all aquaria were loosely covered to minimize the penetration of light (a combination of natural light and a fluorescent source on a 12 h light/dark cycle). We monitored the survival (as time to starvation) of 'starved' settlers every second day for 50 days post-metamorphosis. Settlers were scored as alive if they were still attached to the dish and had a visible lophophore either extended (Fig. 1) or retracted into the zooid. Settlers were scored as dead if they were missing or the lophophore was absent.

Settlers allocated to the 'fed' treatment were treated similarly to 'starved' settlers, except that field-collected filtered seawater was supplemented with equal amounts of the laboratory-grown phytoplankton, *Pavlova lutheri* and *Isochrysis* sp. (a standard food source in laboratory studies of *B. neritina*; Gosselin & Qian 2000) at a total concentration of 10<sup>5</sup> cells mL<sup>-1</sup>. Food and seawater were replaced every third day. This food concentration was high relative to those reported for coastal Australian waters (e.g. McKinnon *et al.* 2003) and ensured that developing colonies could feed *ad libitum* (zooids were seen still feeding at the time of food and water replacement). We monitored the survival (scored as above) and growth (scored as the number of zooids per colony) of 'fed' settlers at 2, 5 and 6 weeks post-metamorphosis for cohort 1 and at 2 and 5 weeks post-metamorphosis for cohorts 2 and 3.

Settlers allocated to the field treatment were transported to the field site in insulated aquaria. There, dishes were bolted to 500 × 500 × 8 mm PVC backing panels (each cohort was deployed on a single panel), which were then suspended facedown (to reduce the effects of light and sedimentation) at a depth of *c.* 1 m below the water surface. We scored the survival and growth of field settlers in the same weeks as reported for laboratory settlers. Survival was scored as above, whereas growth was scored as the number of times a colony had bifurcated along its longest branch (a standard index of size in *B. neritina* that is reliably converted to the number of zooids per colony using equations in Keough & Chernoff 1987). We used this alternative method of scoring growth because counts of individual zooids were not feasible for large colonies in the field.

DATA ANALYSIS

We tested the effect of offspring size on the time to starvation of *B. neritina* settlers in the ‘starved’ treatment using an analysis of covariance (ANCOVA) with cohort modelled as a random categorical effect and larval size as a covariate. We found a non-significant interaction between offspring size and cohort ( $F_{2,56} = 0.52$ ,  $P = 0.59$ ) and therefore examined a reduced model without this interaction.

We explored the relationship between offspring size and performance in the laboratory ‘fed’ and field treatments in two ways. First, we used logistic regressions to examine effects of offspring size on survival at 2 and 5 weeks post-metamorphosis. At week 2, however, ‘fed’ settlers had suffered no mortality in the laboratory; hence, regression models were fitted only to data for field settlers. At week 5, cohorts 1 and 3 had suffered no further mortality in either the laboratory or the field; hence, a regression model was fitted only to data for cohort 2. This model included environment as a fixed categorical effect to compare the effect of offspring size on survival between laboratory and field treatments.

Second, we used repeated-measures ANCOVAs to examine effects of offspring size on subsequent colony size. We initially fitted a full model with environment (fixed effect), cohort (random effect) and offspring size (covariate) as between-subjects effects and time (2 and 5 weeks post-metamorphosis) as a random within-subjects effect. This analysis detected a significant four-way interaction among all effects ( $F_{2,81} = 5.41$ ,  $P < 0.01$ ), indicating that differences in offspring size effects between environments varied according to the cohort and sampling time. Hence, to ease interpretation, we tested offspring size effects for each cohort separately (Quinn & Keough 2002). This approach further allowed us to include an extra sampling time (6 weeks post-metamorphosis) for cohort 1.

OPTIMALITY MODELLING

We used a previously developed optimality model (e.g. Marshall, Cook & Emler 2006; Marshall & Keough 2006) to examine differences in the predicted OOS among experimental environments. The model used significant estimates of offspring size effects from our experimental results and modelled offspring size across a range of values encompassing those observed. This approach assumes that the lines of best fit for our parameter estimates reflect the average strength and form of selection on mothers with respect to offspring size. Like previous models (e.g. Smith & Fretwell 1974; Levitan 1996), ours incorporated a trade-off between offspring size and number,

$$N = \frac{M}{s}, \tag{eqn 1}$$

where  $N$  is the number of offspring produced by a mother with  $M$  resources (an arbitrary value kept constant throughout) and  $s$  the offspring size (estimated as larval volume; see above). To predict the survival ( $B$ ) of a settling larva of a given size ( $s$ ) by the end of the experiment in either the laboratory or the field, we used

$$B = \frac{e^{\alpha s + \beta}}{1 + e^{\alpha s + \beta}}, \tag{eqn 2}$$

where the constants  $\alpha$  and  $\beta$  were generated from any significant logistic regression of larval size on subsequent survival. The relationship between larval size ( $s$ ) and colony size ( $G$ ) in either the laboratory or the field by the experiment’s end was modelled as

$$G = \gamma s + \delta, \tag{eqn 3}$$

where the constants  $\gamma$  and  $\delta$  were generated from a linear regression of larval size on colony size. Given the strong correlation between size and fitness in colonial marine invertebrates (Jackson & Coates 1986; Harvell & Grosberg 1988) and *Bugula neritina* specifically (Keough 1989; Marshall, Bolton & Keough 2003), we next combined eqns (1–3) to estimate maternal fitness ( $\Psi$ ) as

$$\Psi = N \times B \times G \tag{eqn 4}$$

The OOS for each environment and cohort was then taken as the value that maximized maternal fitness. Note that we constrained the minimum size to be 0.027 mm<sup>2</sup> (just smaller than the smallest size observed) and the maximum size to be 0.077 mm<sup>2</sup> (just larger than the largest size observed). For ‘starved’ cohorts, we omitted  $B$  and substituted a linear regression of larval size on time to starvation into  $G$ . Thus, the OOS predicted by this model was that which maximized maternal fitness in terms of the duration of offspring survival.

Results

EFFECTS OF OFFSPRING SIZE ON TIME TO STARVATION IN THE LABORATORY

Offspring size had a significantly positive effect on the time to starvation of *B. neritina* settlers in the laboratory, with settlers that developed from larger larvae taking longer to starve than those that developed from smaller larvae ( $F_{1,58} = 5.84$ ,  $P = 0.02$ ; Fig. 2). This effect was consistent among cohorts ( $F_{2,58} = 0.43$ ,  $P = 0.65$ ; Fig. 2).

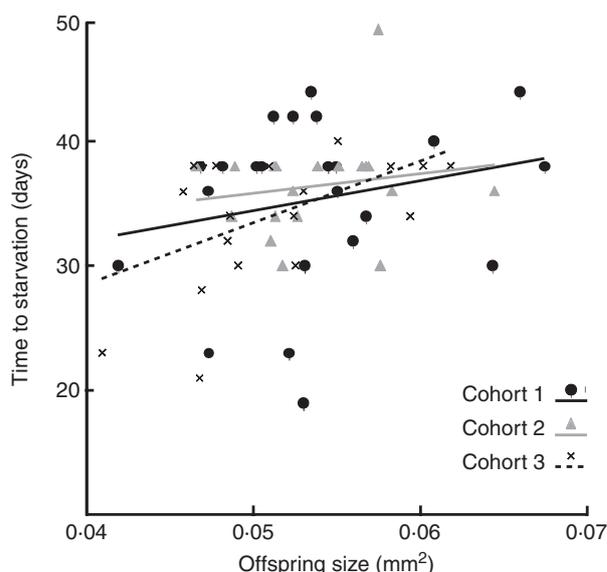


Fig. 2. Effects of offspring size (in mm<sup>2</sup>) on the time to starvation of *Bugula neritina* settlers in the laboratory. Each point represents a single settler and each line shows the regression fitted to these variables for settlers of the same cohort.

## EFFECTS OF OFFSPRING SIZE ON SURVIVAL IN THE LABORATORY VS. THE FIELD

At 2 weeks, colony survival was 100% for all three 'fed' cohorts in the laboratory. In the field, offspring size had a significantly positive effect on colony survival for cohort 1 ( $\chi^2 = 7.94$ ,  $P < 0.01$ , d.f. = 1; Fig. 3), but there was no significant relationship between offspring size and survival at this time for cohort 2 ( $\chi^2 = 0.13$ ,  $P = 0.72$ , d.f. = 1) or cohort 3 ( $\chi^2 = 0.91$ ,  $P = 0.34$ , d.f. = 1).

At 5 weeks, neither laboratory nor field settlers in cohorts 1 and 3 had suffered any further mortality. Mortality was substantial in both treatments for cohort 2, but there was no overall effect of offspring size on survival ( $\chi^2 = 0.07$ ,  $P = 0.80$ , d.f. = 1), nor did the effect of offspring size on survival differ between the laboratory and the field ( $\chi^2 = 1.97$ ,  $P = 0.16$ , d.f. = 1).

## EFFECTS OF OFFSPRING SIZE ON COLONY GROWTH IN THE LABORATORY VS. THE FIELD

Across cohorts, *B. neritina* colonies grew significantly larger in the field than in the laboratory (e.g. Table 1, Fig. 4; note that figures show ln-transformed sizes for field colonies, but untransformed data were analysed). In cohort 1, we detected a significant interaction between time, offspring size and environment (Table 1), which resulted from an increased effect of offspring size on colony growth over time in the laboratory but not the field (Fig. 4a,b). In the laboratory, the effect of offspring size on growth was only marginal early on, but increased over time so that colonies from larger offspring were much larger than colonies from smaller offspring after 6 weeks (repeated-measures ANCOVA for 'fed' settlers alone: time  $\times$  offspring size,  $F_{2,32} = 3.49$ ,  $P = 0.04$ ; Fig. 4a). In contrast, there was no effect of offspring size on colony size in the field (Fig. 4b), perhaps because many colonies from smaller larvae had died after only 2 weeks there.

In cohort 2, colony size was independent of offspring size; alone (offspring size:  $F_{1,25} = 0.27$ ,  $P = 0.61$ ) or in combina-

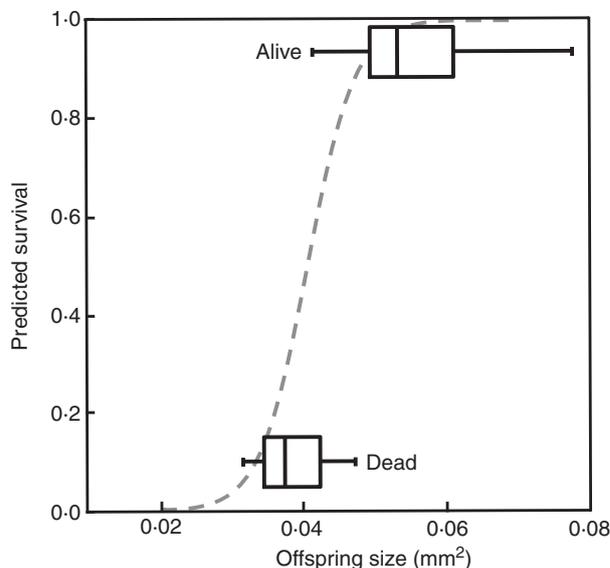


Fig. 3. The effect of offspring size (in mm<sup>2</sup>) on the survival of *Bugula neritina* colonies after 2 weeks in the field (cohort 1 only). The upper box plot represents the size distribution of colonies that survived for 2 weeks and the lower box plot represents the size distribution of colonies that died in this time. The dashed line represents the predicted probability of survival given by logistic regression.

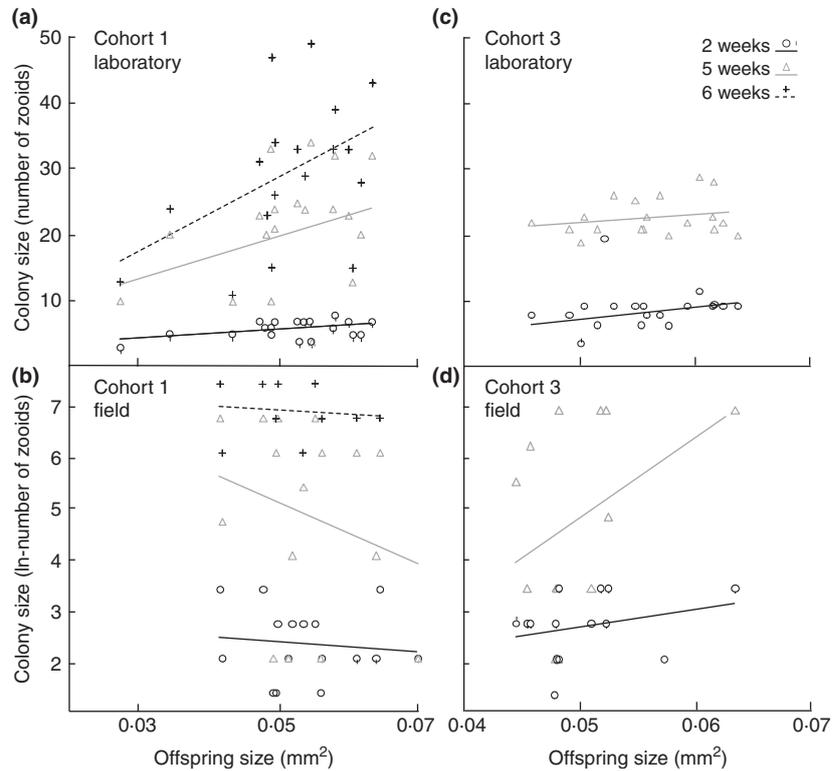
tion with any other factor of interest (offspring size  $\times$  environment:  $F_{1,25} = 0.23$ ,  $P = 0.63$ ; time  $\times$  offspring size  $\times$  environment:  $F_{1,25} = 0.31$ ,  $P = 0.58$ ). Hence, we ran a reduced model without offspring size that detected strong effects on colony growth of experimental environment ( $F_{1,25} = 23.81$ ,  $P < 0.01$ ), time ( $F_{1,25} = 23.48$ ,  $P < 0.01$ ) and time  $\times$  environment interaction ( $F_{1,25} = 21.58$ ,  $P < 0.01$ ). In effect, settlers in the field grew into larger colonies than did 'fed' settlers in the laboratory, and differences in colony size between environments increased over time (see Fig. S1).

In cohort 3, we detected a significantly positive effect of offspring size on colony growth across environments. This effect was stronger in the field than in the laboratory (Table 1,

**Table 1.** Repeated-measures ANCOVA between laboratory 'fed' and field environments comparing the effect of offspring size on the growth of *Bugula neritina* colonies over time for cohorts 1 and 3

Source	Cohort 1				Cohort 3			
	d.f.	Mean square	<i>F</i>	<i>P</i>	d.f.	Mean square	<i>F</i>	<i>P</i>
<b>Between subjects</b>								
Offspring size	1	560.46	3.38	0.08	1	249.72	7.68	<b>0.01</b>
Environment	1	1468.19	8.84	<b>0.01</b>	1	156.82	4.82	<b>0.04</b>
Offspring size $\times$ environment	1	606.95	3.66	0.07	1	244.12	7.51	<b>0.01</b>
Error	27	166.03			29	32.52		
<b>Within subjects</b>								
Time	2	494.85	9.14	<b>&lt; 0.01</b>	1	142.85	4.60	<b>0.04</b>
Time $\times$ offspring size	2	197.54	3.65	<b>0.03</b>	1	226.21	7.28	<b>0.01</b>
Time $\times$ environment	2	495.88	9.16	<b>&lt; 0.01</b>	1	144.85	4.66	<b>0.04</b>
Time $\times$ offspring size $\times$ environment	2	208.69	3.86	<b>0.03</b>	1	223.79	7.20	<b>0.01</b>
Error	54	54.13			29	31.07		

After metamorphosis, colonies were sampled at 2, 5 and 6 weeks for cohort 1, and 2 and 5 weeks for cohort 3 (see text for reduced cohort 2 model). Significant *P*-values are highlighted in bold.



**Fig. 4.** Effects of offspring size (in  $\text{mm}^2$ ) on the subsequent size of *Bugula neritina* colonies in (a) the laboratory for cohort 1; (b) the field for cohort 1; (c) the laboratory for cohort 3; and (d) the field for cohort 3. Each point represents a single colony sampled at 2, 5 or 6 weeks post-metamorphosis (due to large differences between sampling dates, the sizes of field colonies have been ln-transformed for illustration).

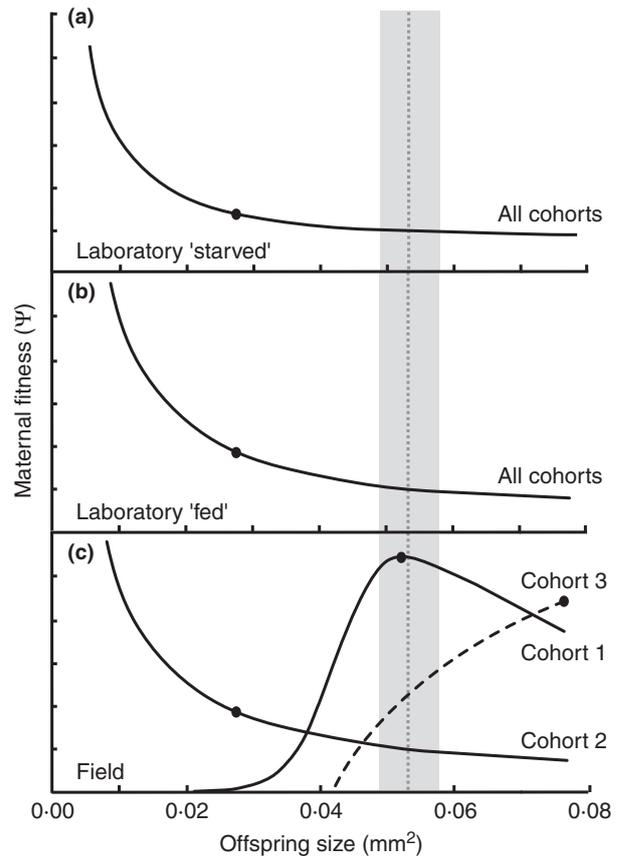
Fig. 4c,d) and varied over time (as was the case for cohort 1; Table 1). Unlike cohort 1, however, the interaction between time, offspring size and environment resulted from an increased effect of offspring size on colony growth over time for field settlers (Fig. 4d) but not laboratory ones (Fig. 4c).

PREDICTED OOSS

Predicted OOSs differed between laboratory and field environments by being highly variable among cohorts in the field and invariant among cohorts in the laboratory. For all cohorts of ‘starved’ settlers in the laboratory, our optimality model predicted maternal fitness to be maximized by producing the smallest possible offspring ( $\sim 0.027 \text{ mm}^2$  in our observed size range; Fig. 5a). Given the trade-off between offspring size and number in our model, this implies that mothers should benefit more from the provisioning of many small offspring than the provisioning of fewer larger ones.

We found similar results for all cohorts of ‘fed’ settlers in the laboratory: maternal fitness was consistently predicted to be maximized by producing the smallest possible offspring (Fig. 5b). This was to be expected for cohort 2 (where no effects of offspring size on colony growth or survival were detected), and also indicates that relationship between offspring size and growth for cohorts 1 and 3, although significant (see above), was too weak for mothers to benefit from increasing offspring size at the expense of fecundity.

Field settlers in cohort 2 also showed little effect of offspring size on either colony growth or survival (see above). Like laboratory settlers in the cohort, this resulted in a predicted OOS close to the minimum observed ( $\sim 0.027 \text{ mm}^2$ ; Fig. 5c). For cohort 1, however, our optimality model param-



**Fig. 5.** Optimality model outputs for *Bugula neritina* in (a) laboratory ‘starved’, (b) laboratory ‘fed’ and (c) field environments. Predicted optimal offspring sizes are indicated by a point on each curve. The dashed vertical line represents the mean observed offspring size; 50% of observed sizes lie in the range of the shaded area.

eterized with survival data from field settlers predicted maternal fitness to be maximized by an offspring size ( $0.052 \text{ mm}^2$ ; Fig. 5c) very close to the observed mean ( $0.054 \text{ mm}^2$ ). Furthermore, the relationship between offspring size and growth for field settlers in cohort 3 was strong enough for our model to predict an OOS at the upper limit of the observed range ( $\sim 0.077 \text{ mm}^2$ ; Fig. 5c), indicating selection for mothers to increase offspring size at the cost of fecundity in this case.

## Discussion

Offspring size usually enhanced the post-metamorphic performance of *B. neritina* in both controlled (laboratory) and natural (field) environments, but this effect varied significantly in strength and consistency among environments and cohorts. Offspring size effects were highly consistent among cohorts of starved settlers in the laboratory (irrespective of cohort, settlers from larger larvae withstood starvation longer than settlers from smaller larvae), but were much more variable among cohorts of laboratory 'fed' and field settlers. Though not often tested, among-cohort variation in offspring size effects may be common in nature (e.g. Marshall & Keough 2008a), where the inherent uncertainty of environmental conditions may expose groups of individuals recruiting to populations at different times (even days apart) to substantially different selection pressures. We were more surprised to find variation in offspring size effects among laboratory 'fed' cohorts (where the relationship between offspring size and post-metamorphic growth was generally steeper for cohort 1 than cohort 3), as the relatively uniform conditions over the experimental duration led us to expect a consistency similar to that of starved settlers in these cohorts. Variation in extrinsic factors seems an unlikely reason for this result. First, our sampling and handling of maternal colonies (see Materials and methods above) was designed to minimize the potential for variation among cohorts due to variation in the external environment (e.g. depth, locality) of their parents. Second, cohorts had similar contemporary environments, growing concurrently for most of the experiment and receiving fresh food and water at identical times from identical sources (note also the similarity of colony sizes across cohorts, attesting to their similar nutrition; Figs 4 and S1). Among-cohort variation in the laboratory could therefore be due to intrinsic factors, such as offspring quality (e.g. energetic content independent of size; McEdward & Coulter 1987; Moran & McAlister 2009) or genetic background, stemming from variation among the groups of parents sampled for each. Such variation may have been suppressed in the more stressful environment of starved settlers.

Across field and laboratory environments, offspring size effects differed in both strength and the performance metric that was affected. For example, larger offspring size enhanced the survival of field settlers in cohort 1 and the growth of field settlers in cohort 3, but affected only the growth of laboratory settlers in either cohort. Offspring size did not enhance growth or survival in either environment for settlers in cohort 2, but did prolong their resistance to starvation in the labora-

tory. When offspring size did affect the same performance measure in both environments (as occurred for growth in cohort 3), the slope of the relationship was *c.* 10-fold steeper in the field. Although this supports the expectation that offspring size–performance relationships should be steeper in harsher environments than in relatively benign ones (Parker & Begon 1986; Sibly & Calow 1986; see also Einum & Fleming 1999; Fox 2000; Marshall & Keough 2008a), what constituted the harsher environment here? On one hand, we may expect organisms to be more stressed (and thus have lower fitness) in laboratory environments that are presumably more alien to them (Herre 1995; Bijlsma & Loeschcke 2005), and we found growth to indeed be lower in the laboratory than the field. On the other hand, laboratory environments are often perceived as more benign than natural ones (Rose, Nusbaum & Chippindale 1996), and our study found survival to be much higher in the laboratory than the field. Regardless of whether performance was measured as growth or survival, however, its relationship with offspring size was steeper in the field compared to the laboratory for two of three cohorts. Thus, we cannot consistently explain the differing effects of offspring size across field and laboratory environments in terms of conventional notions of environmental stress, and the adequacy of 'performance' in defining such stress may differ according to the measure used. Even so, it is clear that offspring size effects estimated for *B. neritina* in the laboratory may not always translate to those expressed in nature.

Perhaps more importantly, we found that the variability of offspring size effects (and hence, of selection on offspring size) in *B. neritina* may be underestimated under controlled conditions. An optimality model parameterized with our results predicted maternal fitness to be maximized by offspring sizes that differed not only between laboratory and field environments, but also among cohorts in the latter. When parameterized with data from laboratory settlers (whether starved or fed), maternal fitness was consistently maximized by producing the smallest possible (and thus, most numerous) offspring. Hence, the effects of offspring size on performance in the laboratory, even when significant, were never strong enough to outweigh the fecundity costs of greater per-offspring investment. When parameterized with data from field settlers, however, maternal fitness was maximized by a different offspring size in each cohort, reflecting selection for an intermediate size in cohort 1 (where observed and optimal offspring sizes coincided; Sinervo *et al.* 1992), for the smallest possible size in cohort 2 and for the largest possible size for cohort 3. Hence, selection on offspring size was constant in the laboratory, but varied among cohorts deployed at largely overlapping times in the same natural environment. This result broadly agrees with Fox's (2000) study on *S. limbatus*, in which selection on egg size varied more for eggs laid on host-plant seeds in the field than for eggs laid on seeds of the same hosts in the laboratory. It suggests that laboratory studies may routinely underestimate natural variability in the offspring size–performance relationship, which may have important consequences for how we view and test ideas about offspring size evolution.

It is worth noting that our laboratory conditions were kept as realistic as possible: *B. neritina* colonies were maintained in field-collected seawater, with light, temperature and orientation matched as best we could to field conditions. Nonetheless, there were potentially major differences between our laboratory and field environments (e.g. food quality, predation pressure), so it is perhaps unsurprising that predictions of the OOS differed between them. Greater effort to incorporate more sources of selection on offspring size into laboratory studies may well improve the match between the optimal sizes predicted under laboratory and field conditions (e.g. Moore & Singer, 1987, Einum & Fleming 1999), although the degree to which this is feasible (or necessary) may vary among taxa according to their life history. More troubling, however, is the differing consistency of the OOS for laboratory- vs. field-reared cohorts. We see no simple solution whereby laboratory environments can be made to effectively mimic naturally stochastic ones, but the reduction of natural stochasticity that inevitably occurs in the laboratory may be the most significant problem with estimating selection on offspring size under such conditions. This is chiefly because theory predicts that variable selection on offspring provisioning may lead to bet-hedging or offspring size plasticity (Forbes 1991; Lalonde 1991; see also Einum & Fleming 2004; and Marshall, Bonduriansky & Bussiere 2008). Hence, focusing on the average strength of offspring size effects, without considering their variability, may fail to provide a comprehensive view of the selection pressures that act on offspring size in the field.

Overall, then, our experiments highlight at least two potential problems with inferring selection on offspring size from estimates of offspring size effects under controlled laboratory conditions: to do so may (i) underestimate such effects (and, by association, the OOS), to the extent that their lack of detection under laboratory conditions may not necessarily exclude their presence in nature; and (ii) underestimate their variability. Previous work (e.g. Einum & Fleming 1999; Fox 2000) has pointed to such issues, but this is the first study to formally examine how the relationship between offspring size and performance may vary within and among field and laboratory environments, and to demonstrate the consequences of such variation for predictions of the OOS. We do not suggest that laboratory estimates of offspring size effects are without merit; rather, we show why they should be treated with caution. Our finding that controlled environments may underestimate the variability of selection on offspring size is a crucial one, emphasizing that mothers face considerable challenges in provisioning their offspring optimally in nature when the optimal size they should produce may be an ever-shifting target.

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## Supporting information

Additional supporting information may be found in the online version of this article.

**Fig. S1.** Effects of offspring size on the subsequent size of *Bugula neritina* colonies in (a) the laboratory and (b) the field for cohort 2.

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