



Does the relationship between offspring size and performance change across the life-history?

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What selection pressures drive the evolution of offspring size? Answering this fundamental question for any species requires an understanding of the relationship between offspring size and offspring fitness. A major goal of evolutionary ecologists has been to estimate this critical relationship, but for organisms with complex lifecycles, logistical constraints restrict most studies to early life-history stages only. Here, we examine the relationship between offspring size and offspring performance in the field across multiple life-history stages and across generations in a marine invertebrate. We then use these data to parameterise a simple optimality model to generate predictions of optimal offspring size and determined whether these predictions depended on which estimate of offspring performance was used. We found that offspring size had consistently positive effects on performance (estimated as post-metamorphic growth, fecundity and reproductive output). We also found that manipulating the experience of offspring during the larval phase changed the way in which offspring size affects performance: offspring size affected post-metamorphic growth when larvae were allowed to settle immediately but offspring size affected survival when larvae were forced to swim prior to settlement. Despite finding consistently positive effects of offspring size, early measures of the effect of offspring size resulted in the systematic underestimation of optimal offspring size. Surprisingly, the amount of variation in offspring performance that offspring size explained decreased with increasing time in the field but the steepness of the relationship between offspring size and performance actually increased. Our results suggest caution should be exercised when empirically examining offspring size effects – it may not be appropriate to assume that early measures are a good reflection of the actual relationship between offspring size and fitness.

Offspring size is a fundamentally variable trait among species, populations and mothers (Williams 1994, Moles et al. 2005, Marshall and Keough 2008), yet the drivers of this variation at all levels remain poorly resolved. One of the major impediments to understanding the evolution of offspring size is the gap between theory and ecological studies of offspring size effects. Biologists have sought to understand offspring size variation for over 60 years and important theoretical and conceptual advances have been made, such that offspring size theory is a fundamental tenet of life-history theory (Lack 1947, Thorson 1950, Bagenal 1969, Moles et al. 2005). Models examining selection on offspring size typically include an offspring size–number tradeoff and an offspring size–performance function, such that maternal fitness is maximised according to an investment strategy that maximises the number of offspring that survive to reproduce (Smith and Fretwell 1974, McGinley et al. 1987, Sargent et al. 1987). In other words, larger offspring typically have higher performance but, because of

their increased cost, producing larger offspring also reduces maternal fecundity. Traditionally, the relationship between offspring size and performance is modelled as curvilinear, such that offspring performance reaches an asymptote for offspring greater than a certain size (Smith and Fretwell 1974, Einum and Fleming 2000). Since Smith and Fretwell's influential paper, the relationship between offspring size and performance has been repeatedly modified for various purposes in order to suit both the life-history of the organism of interest and the way in which offspring size effects manifest (Parker and Begon 1986, McGinley et al. 1987); but, ultimately, selection acts to maximise maternal fitness through the number and the fitness of offspring (Bernardo 1996, Marshall and Uller 2007). There are numerous working definitions of fitness that could be applied but as a generalisation, one of the best estimates of offspring fitness would be the estimated by the number and quality of progeny that those offspring produce themselves (Hunt et al. 2004). As such, offspring size studies should ideally include the effect of offspring size on the total reproductive output of those offspring. Estimating the relationship between offspring size and offspring fitness, however is more challenging for some organisms than others.

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Estimating the offspring size–fitness relationship varies in difficulty depending on the life-history of the study organism. In mammals and birds, where development is direct; offspring are relatively large and relatively few; individual offspring can be tracked, and estimates of the effect of original offspring size on the lifetime performance of offspring (and in some cases, performance grand offspring as well, Clutton-Brock 1991) are possible (Williams 1994). Thus, in well studied mammalian and avian systems, it is possible to estimate offspring fitness rather than some proxy of fitness and as such, field studies in these systems provide some of the best data for offspring size optimality models. In contrast to mammals and birds, most invertebrates produce offspring that go through a larval stage which is typically far smaller, more numerous, and highly dispersive; so for invertebrates with complex lifecycles, estimating the relationship between offspring size and genuine fitness is far more challenging.

For invertebrates with complex life-cycles, most estimates of the offspring size–performance relationship are restricted to early life-history stages and this focus probably reflects the fact that logistical constraints restrict most empirical studies to early stages (but see Plaistow et al. 2007). Traditional estimates of the relationship between offspring size and performance (i.e. those based on early life-history stages) should be retained only if they predict the relationship between offspring size and offspring fitness overall. How likely is such scenario in organisms with complex life-cycles? Even slight changes local environment can strong affect the relationship between offspring size and performance can have significant impacts on the predicted optimal offspring size (Parker and Begon 1986, McGinley et al. 1987, Bashey 2006, Gagliano et al. 2007, Marshall and Keough 2008) and for organisms with dispersive larvae, environments encountered during early life-history stages are often very different to those encountered in later life-history stages. Offspring size effects have also been shown to diminish across the life-history, such that early estimates of effects may result in overestimates of the subsequent optimal size (Heath et al. 1999, Lindholm et al. 2006, Wilson and Reale 2006). More generally, ‘compensatory growth effects’ – whereby initial differences in offspring size are obliterated by increases in the relative growth rate of smaller offspring – suggest that early measures of offspring size effects are likely to overestimate their impact on offspring fitness (Monteiro and Falconer 1966, Gagliano and McCormick 2007a). The problems associated with examining only early life-history stages are exacerbated by the fact that the relationship between offspring size and performance can change across different life-history stages with a negative relationship in one stage but a positive relationship in another (Hendry et al. 2001, Marshall and Keough 2003, Marshall and Bolton 2007). Thus, using estimates of the offspring size–performance relationship based on early life-history stages alone could result in the mis-estimation of not only the strength, but also the direction of the relationship (Hendry et al. 2001). On the other had, using early measures is faster, more convenient and may be the only possible option for some species; and so basing offspring size studies on early life-history stages only has

considerable appeal. In order to do assess the utility of early life-history measures for estimating the relationship between offspring size and performance, a systematic comparison of early offspring size effects to metrics more closely linked with offspring fitness is required. For organisms with complex life-cycles however, examinations of offspring size effects across the entire life-history which include reproductive output, are rare generally and almost never conducted in the field.

Here, we examine the effects of offspring size on a range of offspring performance metrics in the field for a colonial marine invertebrate, the encrusting bryozoan *Celleporaria* sp. In several species of marine bryozoan, offspring size affects performance in both the larval stage and the post-larval stages (Marshall et al. 2008b). Thus, we examined the effect of offspring size on both the larval period and post-metamorphic performance, including post-metamorphic survival, adult growth, fecundity and second generation offspring size (combined, the last two yield total reproductive output – the best estimate of offspring fitness that we could collect). Numerous authors have highlighted the importance of conducting offspring size studies under as realistic conditions as possible (Einum and Fleming 1999, Heath et al. 1999, Fox 2000). While it is possible to replicate field conditions in the laboratory for some organisms (Plaistow et al. 2006), laboratory conditions can result in an underestimation of offspring size effects for others (Einum and Fleming 1999). We therefore conducted our experiments on post-metamorphic performance in the field. Similarly, offspring size effects may be highly context-dependent, changing in direction and magnitude according to the experiences of the offspring (Kaplan 1992, Benton et al. 2005, Marshall et al. 2006, Gagliano and McCormick 2007b, Plaistow et al. 2007, Bashey 2008). Consequently, we examined the effects of offspring size on offspring performance over two experimental runs (to assess temporal variability). In marine invertebrates with a non-feeding larval stage, extending the larval period is reduces post-metamorphic performance, presumably because increasing the amount of time spent swimming decreases the level of energetic reserves available for post-metamorphic growth (Wendt 1998, Marshall et al. 2003b). Furthermore, changes in the length of time that larvae spend swimming prior to metamorphosis can change the relationship between offspring size and post-metamorphic performance (Marshall and Keough 2006). Thus, extending the length of the larval period may affect the offspring size–performance relationship because the energetic costs of larval swimming differ between smaller and larger larvae (Marshall and Keough 2006). Therefore, in a third experiment, we manipulated the time larvae spent swimming prior to settlement and examined the effect of this manipulation on the offspring size–performance relationship. We then used our data from all of our experiments to parameterise a simple offspring size optimality model and we explicitly compared predictions of optimal offspring size based on early offspring size effects to predictions based on the effects of offspring size on reproductive output.

Material and methods

Study species and general methods

We collected mature colonies of the encrusting bryozoan *Celleporaria* sp. from artificial settlement plates suspended from floating docks of East Coast Marina, Manly (153°10'59''S, 27°28'01''E), Queensland, Australia in the austral spring of 2008. We transported colonies to laboratory and maintained them in dark tanks with fresh seawater and aeration for 48 h. We then exposed the colonies to bright light to induce spawning (Marshall et al. 2003a) and larvae were released from ovicells within 15 min of light exposure. Larvae were either measured at this point or allowed to settle immediately (depending on the experiment). Larvae were placed on roughened acetate sheets (200 × 200 mm) in a drop of seawater and allowed to settle in darkness for two h. Once the larvae had settled, settlers were individually marked and identified by circling them with a graphite pencil. Any larvae that had not settled after two h were discarded (almost all settled within 20 min) and settlers were allowed to complete metamorphosis for 24 h at a constant temperature (20°C) in plastic trays filled with fresh seawater.

Measurement of larvae and settlers

We measured larvae using standard techniques developed for measuring bryozoan larvae (Marshall et al. 2003a). Briefly, we placed larvae in a drop of seawater and digitally photographed them under a dissecting microscope. To measure settlers, we allowed larvae settle, develop for 24 h before photographing them from above as in (Marshall and Keough 2004, 2008). Larvae and settlers cross-sectional areas were then estimated with Image tool ver. 3.0 to the nearest square micrometer. For all of our experiments examining post-metamorphic performance, we based our measurements of offspring size on settler size rather than larval size because easier to measure large numbers of stationary settlers than in it is to measure motile larvae. We found a strong relationship between larval size and settler size ($R^2 = 0.57$, $p < 0.01$, Fig. 1) and this relationship was unaffected by the duration of the larval swimming period.

Experiment 1. Does offspring size affect the duration of the larval period?

We were interested in whether larval size affects the length of time larvae spend swimming prior to settlement and because settlement surfaces can affect larval behaviour, we conducted our experiments on two different surfaces. We collected 72 larvae from five different colonies (which were collected from separate backing plates and were, therefore, different genotypes), quickly measured them as described above and randomly allocated them to drops of water placed on acetate sheets. Half of the larvae were allocated to acetate pre-roughened with sand paper and the other half to untreated sheets. We checked settlement every 15 min for two h (after which, all the larvae had attached). Larvae were classed as settled if they were firmly attached to the acetate sheet and could not be removed using a gentle jet of water from a pipette.

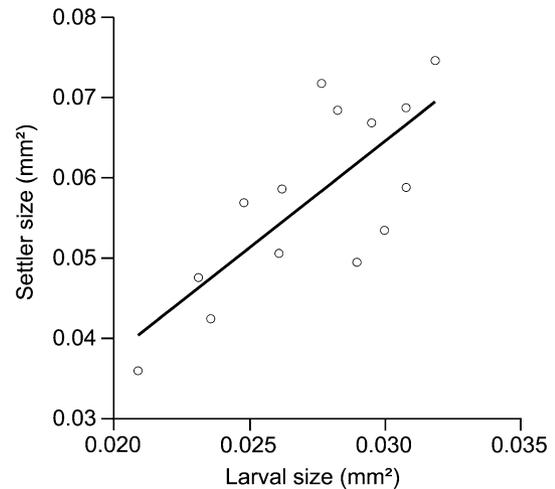


Figure 1. The relationship between larval size and subsequent settler size 24 h after settlement for the bryozoan *Celleporaria* sp. Each point represents an individual larva.

Experiment 2. Does offspring size affect post-metamorphic performance in the field

To test whether offspring size affected colony survival, growth and reproductive output (fecundity and per offspring investment), we settled larvae on acetate sheets and deployed them in the field. The acetate sheets, with four settlers each, were attached with plastic plugs to two 500 × 500 × 8 mm PVC backing panels (four sheets per panel). Plates were hung 1 m below surface, facing down to reduce effects of sedimentation. After two weeks and six weeks in the field, we photographed the colonies to assess colony size. We deployed two runs, 36 settlers (from five different colonies) in the first and 40 (from five different colonies) in the second. We were interested in fecundity of our focal colonies and the quality of the offspring that they produced so after six weeks in the field, we transported the colonies back to laboratory and induced the colonies to spawn and then measured the size of their offspring as described above. For each colony, we counted the total number of offspring that were released and measured 20 haphazardly selected offspring.

Experiment 3. Does the length of the larval period affect the relationship between offspring size and post-metamorphic performance?

Extending the length of the larval period can reduce the amount of resources non-feeding larvae have available for post-metamorphic performance (Pechenik et al. 1998, Wendt 1998). To examine how larval swimming time affected the relationship between settler size and performance, we collected 90 larvae from five different colonies and artificially delayed metamorphosis of half of the larvae for 1 h. Non-delayed larvae were allowed to settle immediately as described above. To delay settlement, we placed larvae in 25 ml plastic vials half-filled with fresh seawater that were kept rotating at low speed in an electronic roller. The constant movement of water inside the tube prevented settlement. After one h, the delayed larvae were offered a suitable settlement surface (sanded acetate sheet) and settled within approximately 20 min. For

each of the groups (delayed and not delayed), we allowed the settled larvae to develop and metamorphose for 24 h as described above, after which the settlers were measured. Importantly, settler size is unaffected by larval duration in this species (pilot analyses: $F_{1,63} = 0.884$, $p = 0.35$; power to detect a 50% change in size: $>95\%$). The acetate sheets were then allocated in the field on three panels as outlined before. We photographed the colonies after one and six weeks in the field and assessed survival and growth. After six weeks in the field, colonies were transported to laboratory and induced to spawn as outlined before. For each colony, we counted the total number of larvae that were released by each colony and measured (when there were sufficient numbers) 10 haphazardly selected larvae.

Data analysis

To examine the effect of offspring size and settlement surface on swimming time, we used ANCOVA where offspring size was a covariate and settlement surface was a fixed factor. We used simple regression to examine the relationship between larval size and settler size. We analysed the effects of offspring size on colony size after two and six weeks using an ANCOVA where settler size was a covariate and panel(run) and run were random factors. Our approach was to first test for an effect of run and its interaction with settler size, where this interaction was not significant, it was removed from the model as it was an experimental convenience (all non-significant terms that were omitted from the final model are presented in Table A1 in Supplementary material Appendix 1, Quinn and Keough 2002). Survival was extremely high (only one colony died) in this experiment so survival was not examined statistically.

To examine the effects of settler size on subsequent reproductive output we used two different metrics: 2nd generation larval number and 2nd generation larval size. We also combined these two elements to calculate totally reproductive output. Both metrics were analysed using again an ANCOVA where parental settler size was a covariate and panel(run) and run were random factors. A similar model reduction approach was taken and non-significant terms that were omitted from the final model are presented in Table A2 in Supplementary material Appendix 1. Where appropriate, differences between panels were assessed with a post-hoc Tukey test.

We could have included acetate(backing panel) throughout our analyses but in preliminary analyses we found that there was no effect of this factor, nor was there an interaction with it and any factor of interest, it explained $<4\%$ of the total variance and was of no biological interest (unpubl.). Thus the assumption that our experimental units (colonies) were varying independently to each other on the same acetate sheets had strong statistical support and as in similar studies (Marshall and Keough 2008), we omitted it from the final model (Quinn and Keough 2002).

To examine the effect offspring size and larval experience on colony size and reproductive output (defined as above) after six weeks in the field, we used ANCOVA where larval duration was a fixed factor, offspring size was a covariate

and backing panel was a random factor. Mortality was high in this experiment and so we examined the effect of larval experience and offspring size with a logistic ANCOVA (essentially, an ANCOVA where the response variable is binary and a logit link function is used, see Quinn and Keough 2002, p. 368 for details), where settlement delay was a fixed factor and settler size a covariate. Again, all non-significant terms that were omitted from the final model are presented in Table A3 in Supplementary material Appendix 1.

Optimality model

We developed an optimality model to examine differences in the predicted optimal offspring size among experimental environments (Marshall and Keough 2008). The model used estimates of significant effects of offspring size on survival, growth, fecundity and reproductive output from our experimental results (Supplementary material Appendix 1), and we varied offspring size across a range of values encompassing those observed. For those factors where interactions were observed between offspring size and experimental run, we used the 'Estimate effects' function in Systat ver. 11 (based on the standard matrix regression formula $B = (X'X)^{-1}X'Y$ to calculate each vector weighting) to estimate the relationship between offspring size and our performance metric of interest for each run and then took the mean estimate of slope and intercept to produce the overall relationship. This approach reflects the fact that selection on offspring size is likely to act according to the average relationship between offspring size and performance (Marshall and Uller 2007). Like previous models (Smith and Fretwell 1974), ours incorporated a tradeoff between offspring size and number,

$$N = \frac{M}{s} \quad (1)$$

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

$$B = \frac{e^{(\alpha s + \beta)}}{1 + e^{(\alpha s + \beta)}} \quad (2)$$

where the constants α and β were generated from a logistic regression of settler size on subsequent survival. The relationship between larval size (s) and subsequent performance (P) was modelled as

$$P = \gamma s + \delta \quad (3)$$

where the constants γ and δ were generated from a linear regression of settler size on either colony size, colony fecundity or reproductive output (depending on which metric was of interest). Thus we were able to explore the impact of using different metrics of offspring performance on predictions of optimal offspring size. We next combined Eq. 1, 2 and 3 to estimate maternal fitness (Ψ) as

$$\Psi = N.B.P \quad (4)$$

To calculate optimal offspring size, we then determined the offspring size at which maternal fitness was maximised for each performance metric of interest.

Results

Experiment 1. Does offspring size affect the duration of the larval period?

Offspring size did not affect larval swimming time, nor did the settlement surface to which they were exposed (mean swimming time on rough surface = 45.47 ± 5.61 min; mean swimming time on smooth surface = 42.41 ± 4.65 min; offspring size effect: $F_{1,57} = 0.02$, $p = 0.879$).

Experiment 2. Does offspring size affect post-metamorphic performance in the field

Settler size strongly affected the size of colonies after two and six weeks in the field (Table 1). Although a significant effect of panels was observed, the relationship between settler size and colony size was always positive. After six weeks in the field, a single panel from run 1 differed from the others (Tukey test, $p < 0.05$), in which growth was higher overall but offspring size still had the same effect on growth. All remaining panels did not differ to each other and were graphically presented as a single set of data (Fig. 2).

Offspring size affected reproduction: colonies that originated as larger settlers produced more offspring themselves than colonies that originated as smaller settlers (Table 2). There was also a positive, though non-significant, relationship between initial settler size and offspring size in the subsequent generation (Table 2). Overall, reproductive output was much higher in colonies from larger offspring compared to colonies from smaller offspring (Table 2). For both fecundity and total reproductive output, there were significant effects of backing plates and interaction between run and settler size. The relationship between offspring size and reproductive output was much steeper in run 1 than run 2, but the relationship of offspring size and subsequent fecundity in both runs was positive. There was a positive relationship between colony growth and reproductive output

Table 1. ANCOVA testing the effect of settler size and panel on colony size for *Celleporaria* sp. after (a) two weeks and (b) six weeks in the field. Note, model is reduced after testing for non-significant random factors and tests of homogeneity of slopes, see text for details. Significant p-values are shown in bold.

Source	DF	MS	F	p
(a) Two weeks				
settler size	1	0.023	5.80	0.020
panel(run)	4	0.017	4.32	0.005
error	43	0.004		
(b) Six weeks				
settler size	1	7.20	13.46	<0.001
panel(run)	4	3.83	7.15	<0.001
error	44	0.54		

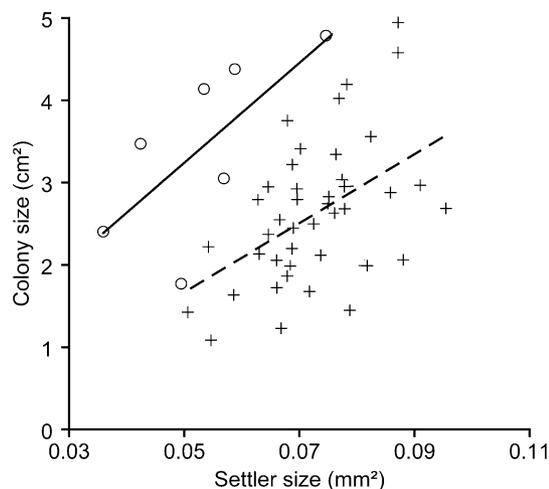


Figure 2. The relationship between settler size and subsequent colony size after six weeks in the field for the bryozoan *Celleporaria* sp. Each point represents an individual colony. Circles and the solid line indicates colonies on a single backing panel that differed significantly from colonies on the remaining backing panels as indicated by crosses and the dotted line.

with colonies that grew more also having a greater reproductive output (measured in terms of both fecundity $R^2 = 0.16$, $p = 0.005$; and total reproductive output $R^2 = 0.15$, $p = 0.0072$).

Experiment 3. Does the length of the larval period affect the relationship between offspring size and post-metamorphic performance?

Larval experience had a strong effect on subsequent survival: colonies from delayed larvae had higher mortality than colonies that had not been delayed as larvae (75% and

Table 2. ANCOVA testing the effect of settler size, panel and run on (a) fecundity, (b) 2nd generation offspring size and (c) total reproductive output of *Celleporaria* sp. after six weeks in the field. Note, model is reduced after testing for non-significant random factors and tests of homogeneity of slopes, see text for details. Significant p-values are shown in bold.

Source	DF	MS	F	p
(a) Fecundity				
settler size	1	7673.34	1.56	0.429
run	1	1389.80	2.13	0.152
run × settler size	1	4901.12	7.51	0.009
panel(run)	3	4159.72	6.37	0.001
error	43	652.99		
(b) 2nd gen. offspring size				
settler size	1	42.72	2.15	0.153
panel(run)	4	48.24	2.43	0.069
error	30	19.86		
(c) Total reproductive output				
settler size	1	55.84	1.44	0.442
run	1	13.75	3.41	0.072
run × settler size	1	38.64	9.59	0.003
panel(run)	3	28.98	7.19	0.001
error	29	4.03		

89% survival respectively). There was a strong interaction between larval experience and settler size on survival after one week in the field: offspring size positively affected survival in the delayed treatment but had no effect on survival in the non-delayed treatment (Wald test: settler size \times delay: $\chi^2 = 8.130$, $p = 0.004$, $DF = 1$, $\rho^2 = 0.364$, Fig. 3). Colony size was not affected by larval experience – colonies from delayed and undelayed larvae reached the same size ($F_{1,49} = 0.01$, $p = 0.991$). For both treatments (delayed and non-delayed), settler size positively affected colony size after 6 weeks in the field ($F_{1,49} = 11.46$, $p = 0.001$) and there was no significant interaction between offspring size and larval experience ($F_{1,49} = 0.109$, $p = 0.743$). No significant relationship between settler size and reproductive output was observed for delayed or non-delayed colonies (Table A4 in Supplementary material Appendix 1).

Predictions of optimal offspring size across metrics

The size of offspring that was predicted to maximise maternal fitness depended strongly on both the experiment and the metric of offspring performance that were used to parameterise our model. Using offspring performance metrics from across experiment 2, we found that the predicted optimal offspring size increased with the use of later performance metrics (Fig. 4). In particular, when we used total reproductive output (fecundity \times 2nd generation offspring size), our model predicted that maternal fitness was maximised by producing offspring that were almost exactly the same size as the mean size of offspring observed for this species (predicted offspring size: 0.072 mm^2 ; observed mean offspring size: 0.071 mm^2 , Fig. 4). When we parameterised our model with the data from our larval experience experiment, the predicted optimal offspring size was 0.058 mm^2 (Fig. 4).

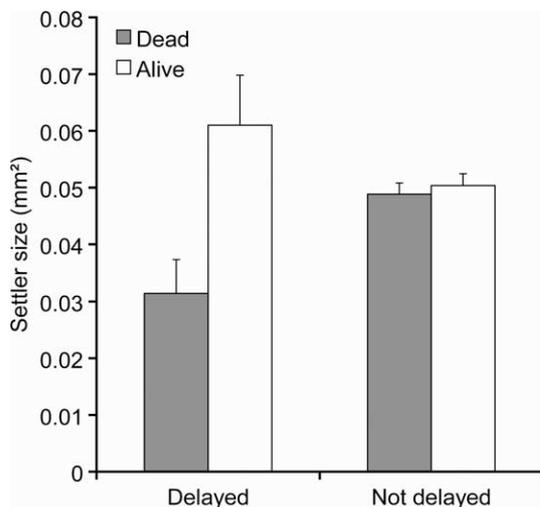


Figure 3. The effect of larval experience and settler size on colony survival after six weeks in the field for the bryozoan *Celleporaria* sp. The bars represent the mean (\pm SE) original size of settlers that lived (open bars) or died (shaded bars) as colonies after six weeks in the field. The set of bars on the left represent colonies that experienced a delay as larvae and the set of bars on the right represent colonies that were allowed to settle immediately.

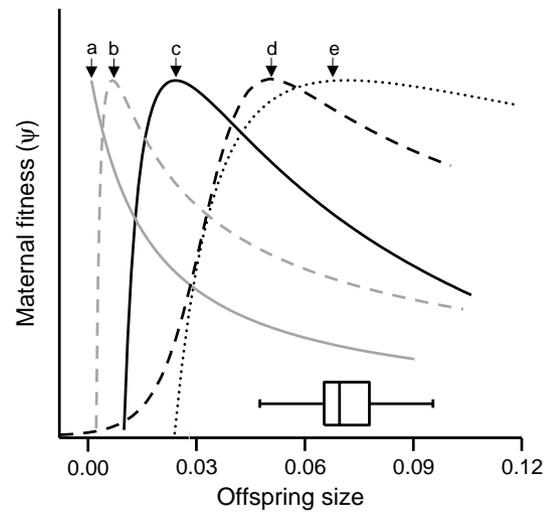


Figure 4. The predicted relationship between offspring size and maternal fitness (ψ) based on an optimality model was parameterised with empirical data. Each line represents a different offspring size-maternal fitness function where the offspring performance metric used to generate the function was based on; (a) colony size after two weeks in the field; (b) colony size after six weeks in the field; (c) fecundity after six weeks in the field; (d) colony that had experienced delays as larvae, (e) reproductive output (fecundity \times 2nd generation offspring size) after six weeks in the field. The box plot at the bottom of the panel indicates the size distribution of ‘wild’ offspring throughout the study period. Note that only the offspring size-maternal fitness function based on reproductive output predicts an optimal offspring size that is close to the observed distribution of offspring sizes in the field.

Discussion

We found strong, positive effects of offspring size on post-metamorphic performance across a number of different performance metrics in the bryozoan *Celleporaria* sp. Larger offspring generally became larger colonies, with greater fecundity and greater reproductive output overall. These findings are in keeping with a growing list of studies that show that offspring size influences performance across the metamorphic boundary in marine invertebrates with complex life-cycles (Emlet and Hoegh-Guldberg 1997, Moran and Emlet 2001, Marshall et al. 2003a), but is one of the few to link offspring size with subsequent reproductive output in the field for any organism. When we used our empirical data to parameterise our simple optimality model, our predictions of optimal offspring size differed depending on the metric that was used. We also found that the effects of offspring size differed in type and strength depending on the experience of the offspring as larvae.

The relationship between offspring size and performance was highly dependent on larval experience. When larvae were allowed to settle immediately, offspring size affected post-metamorphic growth but when larvae were forced to swim for an extended period, offspring size affected post-metamorphic survival instead. Numerous studies have shown that extending the larval period reduces post-metamorphic performance in marine invertebrates with non-feeding larvae (Pechenik et al. 1998, Pechenik 2006). Extending the larval period presumably reduces post-metamorphic performance

because it depletes the amount of energetic reserves available to settlers to use after metamorphosis (Wendt 2000, Bennett and Marshall 2005). It appears that the larval experience, as well as the adult environment, can dramatically change the way in which the benefits of increased offspring size manifest in later life-history stages and thus change the offspring size–performance relationship.

Our optimality model predicted that maternal fitness would be maximised by very different offspring sizes depending on the metric of post-metamorphic performance that we used. Laboratory studies have found that early estimates of offspring size effects overestimate their influence relative to later effects (Heath and Blouw 1998, Lindholm et al. 2006, but see Plaistow et al. 2006) and, more generally, that compensatory growth reduces the influence of maternal effects (Monteiro and Falconer 1966, Wilson and Reale 2006). In contrast, we found the benefits of increased offspring size were compounded over time. The underestimation of offspring size effects using early measures may relate to the allometric offspring size effect that we observed. To explain, across the smallest and largest settlers used in our study, there was a 53% difference in size: after two weeks in the field, this initial difference in offspring size had translated into a $\sim 100\%$ difference in colony size. After six weeks in the field, the difference in size between colonies from the smallest and largest settler was 200% and this differential increased to 225% for reproductive output.

Interestingly, the increase in the slope of the relationship between offspring size and performance across different metrics and over time occurred despite an associated increase in the influence of ‘noise’ (genetic and environmental effects) on those metrics over time. The amount of variation explained by offspring size effects on post-metamorphic performance alone (calculated from raw regressions of offspring size on various elements of performance) dropped from $\sim 23\%$ for colony size after two weeks in the field to 17% of the variation in colony after six weeks in field and down to 10% for reproductive output overall. Thus, while the ability of offspring size to explain variation in performance relative to environmental effects diminished over time (as has been shown for other species: Heath et al. 1999, Beckerman et al. 2006), the effect size of offspring size increased. This finding both supports and complicates the view that the strength of maternal effects decreases with ontogeny (Wilson and Reale 2006). Our finding supports the view that the amount of variance in offspring performance that is explained by maternal effects decreases over time. Surprisingly, however, a strong relationship (where ‘strength’ is estimated by the slope of the relationship between offspring size) persists and actually strengthens over time – a hitherto unexpected effect that directly contradicts the suggested role of compensatory growth effects (Monteiro and Falconer 1966). Whether the lack of a compensatory growth effect in our study is a property of colonial organisms more generally (given that colonial organisms in particular exhibit strong, allometric growth, Sebens 1987) or is a product of examining offspring size effects in the field rather than the laboratory remains to be tested (Fox 1997, 2000). It is also important to note that a limitation of this study is that it examined phenotype only (Lindholm et al. 2006). A crucial next step will be to examine whether the effect of offspring size we

observed can be estimated within a quantitative genetics framework so that we can further understand how selection will shape the evolution of offspring size in this species.

Despite finding consistent, positive effects of offspring size on each metric of performance in our first field experiment, the use of earlier measurements of performance consistently underestimated the benefits of offspring size relative to using total reproductive output. Our finding that using proxies for fitness based on early history traits (e.g. initial growth) results in the underestimation of predicted optimal offspring size (relative to later measures) has some interesting implications for offspring size studies. Our results challenge the implicit assumption that effects of offspring size on initial performance will reflect the magnitude of these effects on later performance (an assumption that has been made in numerous studies, e.g. Marshall and Keough 2008). Furthermore, studies that model the offspring size–performance relationship as asymptotic (Smith and Fretwell 1974) may underestimate the benefits of increased offspring size at the upper range (i.e. once a fitness value of ‘1’ is reached, no further benefit is possible). Future studies should consider that offspring size effects can compound via multiplicative effects on growth and reproduction rather than diminish over time.

It is important to note that our predictions of optimal offspring size were highly context-dependent even when we based our performance measures on reproductive output and this finding highlights the challenge that mothers face when provisioning their offspring. When we parameterised our model using final our reproductive output data, the model predicted an optimal offspring size that was very close to the observed offspring size in the field. However, the relationship between offspring size and performance in our experiments varied due to larval experience and also depended on the date that offspring were released (recall that there was a significant settler size \times experimental run interaction) so the actual optimal offspring size may be an ever-shifting target of selection and this may explain why offspring size exhibits so much variability both among and within individual batches of offspring (Marshall et al. 2008b).

Our goal here has not been to critique previous studies that have examined offspring size effects in the early parts of the life-history. Indeed, we suspect that in many systems, offspring size effects are restricted to the early stages alone and as such, studies that focus on these stages are appropriate. Nevertheless our results suggest future studies should not necessarily assume that early effects of offspring size reflect the direction and magnitude of the relationship between offspring size and offspring fitness in the field and that further tests of this critical relationship are necessary if we are to make progress in understanding the ecology and evolution of offspring size.

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Supplementary material (available online as Appendix O17725 at <www.oikos.ekol.lu.se/appendix>). Appendix 1.