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The Relationship between Offspring Size and Performance in the Sea

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ABSTRACT: The historical focus on offspring size has been to explain variation among populations, but there have been few attempts to determine whether variation is greatest at population scale. Offspring size variation is typically viewed as an adaptive response to changes in the relationship between offspring size and performance, yet direct tests remain elusive. We partitioned natural variation in offspring size for a marine invertebrate, *Watersipora subtorquata*, at a range of spatial and temporal scales across southeastern Australia, and we estimated the relationship between offspring size and performance at each population and time. There was significant variation in offspring size among populations, but regional differences explained only ∼25% of the observed variation, suggesting that there should be a greater focus on small-scale variation in offspring size. We used our data to parameterize an optimality model to generate predictions of offspring size among different populations and times. Differences in the relationship between offspring size and postmetamorphic performance (and therefore changes in size of offspring that were predicted to maximize maternal fitness) among populations and times were associated with differences in offspring sizes among those populations and times. We suggest that interpopulation variation in offspring size can be an adaptive response to local conditions, but the optimal offspring size is surprisingly dynamic.

Keywords: egg size, maternal effects, transgenerational plasticity.

Offspring size is one of the most important and well-studied traits in evolutionary ecology (Lack 1947; Vance

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1973*a*; Smith and Fretwell 1974; Stearns 1992; Williams 1994; Bernardo 1996). Offspring size affects the fitness of mothers and offspring simultaneously, but selection acts to maximize maternal fitness with regard to the per capita provisioning of offspring (Smith and Fretwell 1974; Bernardo 1996). Because the resources available for reproduction are limited, it follows that mothers can make either a few well-provisioned offspring or more numerous poorly provisioned offspring (Smith and Fretwell 1974; Parker and Begon 1986). The optimal balance between these extremes is determined by the relationship between offspring size and fitness (Smith and Fretwell 1974). Generally, a steeper relationship between offspring size and fitness increases the benefits of producing larger offspring, and a shallow relationship favors the production of smaller offspring (Smith and Fretwell 1974). For any single set of environmental conditions, a single optimal offspring size is predicted to maximize maternal fitness (Einum and Fleming 2000; Hendry et al. 2001). In nature, however, offspring size varies surprisingly in space (among and within populations, females, and individual broods) and in time for any one species (Bernardo 1996). This intraspecific variation in offspring size raises two fundamental questions: does among-population variation in offspring size represent an adaptive response to local conditions, and is this variation important? Despite 60 years of offspring size studies (Berrill 1935; Lack 1947; Bagenal 1969; Crump 1981), we have few data with which to address these questions.

One of the more striking aspects of offspring size is the variation observed among populations of the same species. For a range of taxa, different populations produce very different offspring sizes (e.g., Barnes and Barnes 1965; Bridges and Heppell 1996; Johnston and Leggett 2002; Hendrickx et al. 2003). A number of explanations for among-population variation have been invoked, but almost invariably, it is viewed as an adaptive response by mothers to the environmental conditions that their offspring will experience (e.g., George 1995; Hendrickx et al. 2003; Kokita 2003; Olsen and Vollestad 2003). The argument is that the relationship between offspring size and

fitness differs among populations, affecting the optimal offspring size. Similarly, seasonal variation in offspring size is also commonly interpreted as an adaptive response to anticipated changes in the environment in which the offspring will live (Brody and Lawlor 1984; Landa 1992; Lips 2001).

Although much discussion focuses on variation among populations, it is not clear that it is the scale at which offspring size is the most variable. There is variation in offspring size at all scales, but formal partitioning of variation in both space and time is rare. Bernardo (1996) highlighted the lack of formal variance partitioning as a major gap in our understanding of offspring size, and he lamented the lack of studies that systematically address offspring size variation at a range of levels; little has changed since his review. Identifying the scales on which offspring size varies is necessary to focus our search for the factors behind any adaptive maternal provisioning/allocation.

Despite the numerous studies that assume that (among-population, seasonal) variation in offspring size is an adaptive response, there are very few direct tests of this assumption. Direct tests require estimations of the relationship between offspring size and fitness at different populations/times (or at least offspring performance, i.e., key elements of the phenotype that are likely to be correlated with fitness, e.g., size, reproductive output). However, it is rarely possible to observe the performance of individuals in the field and even more difficult to replicate these observations among populations. Thus, the most common approach is to indirectly measure some relevant environmental variables and determine whether they are correlated with variation in offspring size among the population (Johnston and Leggett 2002; Hendrickx et al. 2003). For example, in an elegant study on the seed beetle *Stator limbatus*, Fox (2000) showed that in trees with seeds that were more resistant to boring, mothers produced larger eggs than they did in trees with less resistant seeds. Larger offspring perform better than smaller offspring on more resistant seeds, and so it was concluded that differences in offspring size among trees was adaptive (Fox 2000). The use of environmental correlates to infer adaptive variation in offspring size has also been extended to interspecific comparisons, and the differences we observe among species are commonly explained using such an indirect approach (e.g., Levitan 2002; Moles et al. 2004; Moran 2004; Martin et al. 2006).

While an indirect approach is a useful one, it has several potential problems. First, it essentially assumes that the observer is measuring all the relevant (from the perspective of provisioning mothers and their offspring) environmental variables and is doing so over the appropriate timescales. The complete characterization of all environmental variables is feasible in some systems, but in others, it is impossible to measure all of the relevant variables and the subtle interactions between them. Incomplete characterization of the relevant variables will be particularly common when offspring size can affect multiple life-history components, potentially in counterintuitive ways (Kaplan 1992; Hendry et al. 2001; Marshall and Keough 2006).

The second potential problem is that environmental variables that may affect the offspring may also affect the mother. Maternal phenotype can change the costs and benefits of producing different-sized offspring; for example, larger mothers may be able to secure better habitat for their young to provision larger young more efficiently, resulting in larger offspring sizes being favored (Venable 1992; Bernardo 1996; Hendry et al. 2001; Sakai and Harada 2001). Offspring sizes may therefore differ between populations because of differential selection on the maternal phenotype rather than on the relationship between offspring size and performance.

An alternative means of determining whether interpopulation or interseasonal variation in offspring size represents an adaptive response to changes in the relationship between offspring size and fitness is to measure the relationship under field conditions at many populations or times. Lifetime fitness is extremely difficult to measure in the field, and thus, the measure of some aspect of performance, while not ideal, is a pragmatic alternative. The direct measurement of the relationship between offspring size and performance in the field therefore avoids the problem of incompletely characterizing the relevant environmental variables, and it isolates the performance of offspring from the phenotype of the mother. Thus, this direct approach avoids the two problems outlined above. We can find few such data sets. In a rare exception, Heath et al. (2003) showed that farmed salmon produce smaller offspring than their wild ancestors and that the relationship between offspring size and performance appears to be weaker in farmed populations than in wild populations. Nevertheless, examples from natural populations in the field remain elusive.

Here we seek to address two fundamental gaps in our knowledge of offspring size ecology and evolution: the scales on which offspring size varies and whether observed interpopulation variation in offspring size is adaptive. We address these gaps by describing variation in offspring size and size-specific performance in the field across a range of spatial (hundreds of kilometers to centimeters) and temporal (across seasons) scales for a colonial marine invertebrate, the bryozoan *Watersipora subtorquata*. *Watersipora* is an excellent candidate for the study of adaptive offspring size variation. The size of offspring that naturally settle in the field can be accurately and nondestructively estimated by measuring the size of settlers (Marshall and

Keough 2003*b*). The adults are sessile and colonial, so survival can be measured simply as presence/absence and colony size can be measured accurately by counting the number of feeding zooids in a colony (Marshall and Keough 2004). Finally, offspring size is correlated with performance in the field (Marshall and Keough 2004). Thus, *W. subtorquata* allows the measurement of offspring size under natural conditions and estimation of the relationship between offspring size and performance in the field, a crucial component of any offspring size study (Fox 2000). We used the data on size-specific performance from our field studies to generate predictions of optimal offspring size at a range of scales, and we compared performance-based predictions of offspring size with the observed sizes in the field.

Material and Methods

Study Organism

Watersipora subtorquata is an encrusting bryozoan and one of the commonest members of the "fouling community" on man-made structures along the south and east coasts of Australia. It broods its larvae for approximately 2 weeks, whereupon the nonfeeding larvae are released and spend only a short time in the plankton (minutes to hours; Marshall and Keough 2003*b*). Importantly, settler size is correlated with larval size, and the correlation is independent of swimming duration in *W. subtorquata* (Marshall and Keough 2003*b*). While it has been suggested that size is a poor reflection of maternal investment in marine invertebrates, we review the available evidence and show that offspring size (rather than energetic content) is a good measure (Marshall and Keough 2007).

Study Sites

We did our experiments in two different regions, Melbourne and Sydney, with two sites within each. All the sites shared a number of features: they all sit in larger bays, and they are relatively sheltered from the prevailing weather conditions either by a man-made breakwater or natural headlands. The fauna in each site is very similar and includes bryozoans, such as several species of *Bugula*, *W. subtorquata*, and ascidians, including didemnids and botryllids and several solitary species, and serpulid polychaetes and barnacles (for a detailed description, see Keough and Raimondi 1995). The different regions differ considerably in seawater temperatures, with Melbourne temperatures ranging from ~12° to ~23°C in winter and summer, respectively, and Sydney temperatures ranging between ~15° and ~24°C.

In Melbourne, we did experiments at two sites, the St Kilda Yacht Marina (37°51′48.52″S, 144°57′55.61″E, hereafter referred to as "St Kilda") and Williamstown Workshops Pier (37°51′39.54″S, 144°54′28.70″E; "Williamstown"). In Sydney, the sites were Kirribilli Yacht Marina (33°50′49.58″S, 151°13′10.19″E; "Kirribilli") and Clontarf Yacht Marina (33°48′16.25″S, 151°15′10.30″E; "Clontarf"). While natural dispersal data are lacking, the short dispersal period of *W. subtorquata* larvae suggests that strong connectivity among sites over ecological timescales is unlikely (Marshall and Keough 2003*b*). Overall, our experiments ran from March to December 2005, but the duration of the study periods in each region varied (table A1 in the online edition of the *American Naturalist*). In Sydney, our experiments ran from March to June 2005, but in Melbourne, they ran from March to December 2005. Throughout the study, for any one region, experiments were deployed simultaneously at each site on the same day, but the dates of deployment differed between regions.

Experimental Methods

To sample the size and number of settlers and monitor their subsequent performance, our experimental equipment and protocol were the same at all sites. We used black Perspex (similar to Plexiglas) settlement plates (100 mm × 100 mm × 6 mm) that had been roughened with sandpaper to encourage settlement. We affixed the settlement plates to PVC backing panels (440 mm × 440 mm × 8 mm) with stainless steel bolts (16 plates per backing panel). At each site, the plates were suspended at a depth of ~1.5 m below the mean low-water mark either from the pier itself or from a line strung between two pilings (10 backing panels per site in Melbourne and five backing panels per site in Sydney). We should note that our design limited our sampled population to settlers on new, bare space rather than on any surface. Nevertheless, we are confident that such bare space accurately represents at least one of the habitats into which *W. subtorquata* settles. Each experimental run lasted 14 days. Plates were deployed on the morning of day 1; settlement of *W. subtorqata* (and other species) then occurred over that day and through until the following morning. On day 2, the plates were retrieved and returned to the laboratory, where the *W. subtorquata* settlers from day 1 were recorded, using a digital camera attached to a microscope (×40 magnification). Any individuals that settled on day 2 had only recently commenced metamorphosis and were easy to recognize and disregard. To identify each settler, we used a 10 × 10 grid system on each settlement plate, and we recorded the settlement plate and the grid reference for each settler. In the rare event that two settlers were in the same grid reference on the same settlement plate, one was randomly selected and removed. At the end of day 2, the

settlers were returned to the field and reattached to the backing plates. On a few occasions in Melbourne when the density of settlement was very high, requiring many settlers to be measured, measurement in the laboratory took longer and we had to redeploy on day 3 rather than day 2. The travel time between the field sites and laboratory varied between 20 min and 1 h for each of the sites.

From day 2 to day 14, the settlers were left in the field to grow into colonies. On day 14, we retrieved the colonies, and back in laboratory, we measured colony survival and size. Colonies were classed as "alive" if they were present and clearly had some living zooids; they were classed as "dead" if zooids were absent or all the zooids were blackened and showed no signs of feeding. To measure colony size, we recorded the number of feeding zooids in each colony with a digital camera attached to a microscope (× 10 magnification). It should be noted that survival to 2 weeks is a good predictor of longer-term recruitment success in *W. subtorquata* (D. J. Marshall, unpublished data).

We were also interested in how much variation in offspring size there was within and among colonies. To test this effect, we brought field-collected colonies into the laboratory, induced them to spawn using standard techniques (Marshall and Keough 2004), and measured the resultant offspring as above. We measured 10 offspring each from 15 colonies from Williamstown only.

Data Analysis

Variation in Offspring Size. To examine variation in settler size among regions, sites, and experimental runs, we used two analyses. We first pooled runs within months for months when there was settlement in all of the sites across both regions (March, April, May, and June). For the regional analysis, the design was a three-factor, partly nested design, with regions and sites nested within regions and crossed with months. All factors were random. For the regional analysis, because all factors were random, we used quasi-*F* ratios (following Winer et al. 1991, pp. 374–377; Quinn and Keough 2002) to test the effect of region in the partly nested analysis.

For Melbourne only, we also examined offspring size variation at the level of individual runs across both sites, using a factorial design where sites and times were fully crossed (orthogonal). We could not repeat these analyses for Sydney because settlement in Kirribilli was sporadic.

Our analysis was not perfectly balanced, with more replicates in Melbourne than Sydney. In unbalanced designs, restricted maximum likelihood (REML) can provide more reliable estimates of variance (Quinn and Keough 2002), so we used the SAS MIXED procedure with REML to estimate variance components for both the among- and

within-region analyses. We also used this analysis to supplement our findings on the significance of factors in the initial ANOVA model, using log-likelihood ratios to compare full and reduced models. Because our data could be considered a time series, we also considered using a function-valued approach; however, there was no covariance structure across sampling times and, thus, no improvement in model fit, so we did not include these analyses here.

Effects of Offspring Size. Offspring size ranges were not completely overlapping between Sydney and Melbourne, and therefore, we examined the effects of offspring size among the different regions with separate analyses. Because there was little settlement in Kirribilli, we could examine the relationship between offspring size and performance across both sites for only three runs in Sydney: March 8, April 10, and May 6. Only one settler died at Kirribilli, so the effects of offspring size on survival at this site were not examined. The effects of offspring size for individual experimental runs were calculated using logistic regression of colony survivorship on initial size and simple linear regression of colony size on offspring size. Effects of offspring size were compared within regions using ANCOVAs (logistic and least squares, for mortality and growth, respectively). For each, we first tested whether there was a significant interaction between offspring size and the categorical factors (otherwise known as a homogeneity-of-slopes test). Where there was no significant interaction, it was removed from the model, and the results of the reduced model are presented.

Scales of Replication and Pooling. Across our experiments, we simplified our statistical models where appropriate by dropping settlement plate and backing panel effects. Such an approach is advocated when the effect is of no biological significance; it is an experimental convenience, and a Type II error is unlikely (Winer et al. 1991; Quinn and Keough 2002). As a conservative measure, we used $\alpha = 0.25$ as our criterion for the dropping of terms from the model. We report the dropped terms in table A2 in the online edition of the *American Naturalist*. In no instance was a there a significant effect of either settlement plate or backing panel.

Optimality Modeling

To generate predicted optimal offspring sizes for any single run, we used the methods outlined by Marshall et al. (2006) and Marshall and Keough (2006). As in other models, the model contained a size-number trade-off:

$$N = \frac{M}{s^{1.5}}, \qquad (1)$$

where N is the number of larvae produced by a mother with M resources and s is the size of each larva (we used $s^{1.5}$ because we used area as our measure rather than volume). Survival of the settlers for 2 weeks was modeled as a simple logistic relationship between offspring size and predicted survival (Marshall et al. 2006):

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

where α and β were both constants generated from the logistic regression for the relationship between settler size and subsequent survival (see fig. 1 for an example of a logistic relationship between size and survival). The relationship between settler and colony size was given by

$$G = s\gamma + \delta, \qquad (3)$$

where γ and δ were both constants generated from the linear regression of settler size and colony size after 2 weeks. Given that colony size is strongly correlated with fitness in colonial marine invertebrates (Sebens 1982, 1987; Marshall et al. 2003), maternal fitness ψ is then given by combining equations (1)–(3) to produce

$$\Psi = NB \times G. \qquad (4)$$

We then calculated optimal offspring size (i.e., settler size that maximized maternal fitness) for each run at each site. It should be noted that we constrained the minimum offspring size in the model to be 0.05 (slightly smaller than the smallest settler that was observed). When there is no relationship between offspring size and performance, the predicted offspring size that maximizes maternal fitness is infinitely small.

Because there was low settlement in some runs, it was impossible to distinguish between Type II errors and instances when there was genuinely no relationship between offspring size and performance. Thus, we pooled offspring size performance estimates at two levels: (i) region and (ii) sites across seasons (summer and winter). These scales of pooling were used because differences in offspring size occurred among Sydney and Melbourne (see "Results") and there was significant variation in offspring size (and subsequent growth) between the warmer and cooler months and at each site. Because we were concerned that larger-scale regional effects could have been driving the patterns we observed, we also used an alternative approach, where we used our model predictions to predict any change in offspring size from winter to summer in
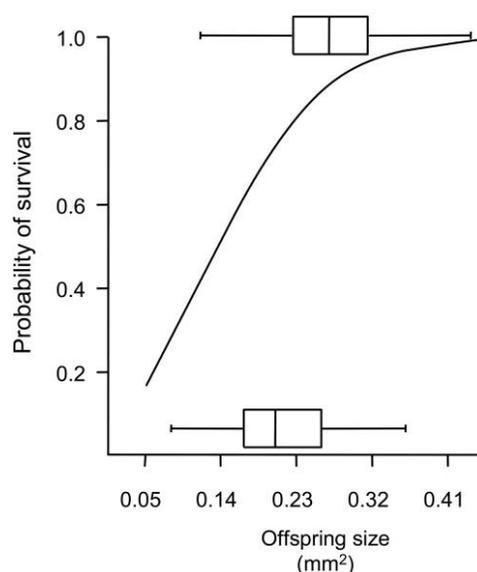

Figure 1: Relationship between *Watersipora subtorquata* offspring size and survival after 2 weeks in the field for settlers in the Melbourne region; data are pooled across experimental runs. The upper box plot represents the size distribution of settlers that survived for 2 weeks, and the lower box plot represents the size distribution of settlers that died within 2 weeks. The line represents the predicted probability of survival that was back-calculated from the logistic regression equation.

each site. We then compared this predicted change with the observed change in offspring size that we examined across seasons. This effectively distilled our data to four points, but it did have the advantage of avoiding any confounding effects of site or region because it compared changes within sites across times.

Results

Variation in Offspring Size

Offspring size in *Watersipora subtorquata* was highly variable, with size ranging from 0.06 to 0.553 mm² and a coefficient of variation of 29% overall. Most (~70%) of the variation in offspring size occurred at scales other than region and appeared to occur among larvae within a single settlement period within a single site (table 1; fig. 2). For example, on a single settlement plate in Melbourne, one settler was three times the size of another. Despite variation at lower spatial scales, there was a significant difference in settler size between regions. Melbourne offspring were, on average, 40% larger than those in Sydney. Within regions, the majority of variation in offspring size was in individual experimental runs in both Melbourne and Sydney (table 2). There was also significant variation in offspring sizes over time across different sites in both regions. Offspring

sizes tended to be larger over the summer months, but there were also interactions between site and time. Further exploration of the Melbourne data showed that there was little variation among sites, times, or panels and that most of the variation in offspring size could not be explained by our model (table A3 in the online edition of the *American Naturalist*). The size of offspring varied significantly among Williamstown colonies ($F = 6.126$, df $= 13, 135$, $P < .001$), but most (66%) of the variation occurred among larvae from individual parent colonies.

Effects of Offspring Size

In Melbourne, offspring size strongly affected subsequent performance, with larger offspring surviving better and growing more as juveniles than smaller offspring. For example, (temporarily ignoring the effects of run) in Melbourne, smaller offspring (e.g., <0.14 mm^2) had less than a 50% chance of surviving, whereas offspring that were larger (e.g., >0.23 mm^2) had more than an 80% chance of survival (values back-calculated from logistic regression coefficients; fig. A1 in the online edition of the *American Naturalist*). Similarly, in summer, smaller offspring (using the same size classes as above) never grew into colonies larger than six zooids after 2 weeks, but larger offspring could develop into colonies with >20 zooids in the same period of time (figs. 3, A3 in the online edition of the *American Naturalist*).

While there were strong effects of offspring size on performance in Melbourne, these effects were highly variable in terms of the specific relationship between size and subsequent performance. When all the data from Melbourne were combined, there were significant interactions between offspring size, run, and site on both survival (Wald test, site × run × offspring size: $\chi^2 = 4.98$, $P = .026$, $\rho^2 = 0.161$) and growth (table 3). In each case, the interaction was driven by the presence of an effect of offspring size in some sites/runs but an absence of an effect in others (figs. A1, A2 in the online edition of the *American Naturalist*).
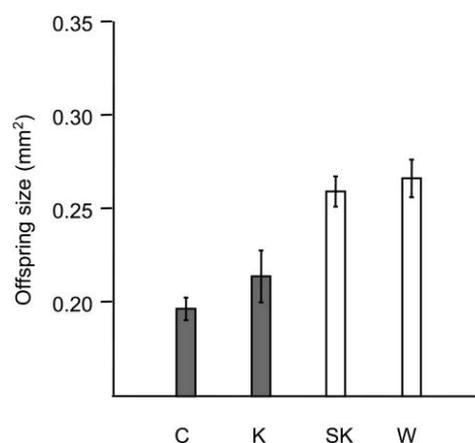

Figure 2: Natural variation in *Watersipora subtorquata* offspring sizes in the field at two different regions (Melbourne and Sydney) and two different sites within each region (C = Clontarf, K = Kirribilli, SK = St Kilda, W = Williamstown). Data are pooled across all experimental runs. The shaded bars represent mean offspring sizes ($\pm$ SE) in the Sydney region, and the open bars represent mean offspring size ($\pm$ SE) in the Melbourne region.

In Sydney, the relationship between offspring size and performance was weaker but was also more consistent among runs. Looking across all runs for Clontarf, there was an effect of settler size on subsequent survival (run × offspring size: $\chi^2 = 0.07$, $P = .789$; run: $\chi^2 = 2.42$, $P = .119$; offspring size: $\chi^2 = 6.6$, $P = .013$). However, there was no interaction between offspring size and run and site on subsequent growth, either alone or in combination, and no effect of offspring size overall (offspring size × run × site: $F = 0.62$, df $= 2, 106$, $P = .54$; offspring size × run: $F = 0.7$, df $= 2, 108$, $P = .49$; offspring size × site: $F = 0.65$, df $= 1, 108$, $P = .42$; table 4). Looking within Clontarf only, there was an indication that the relationship between offspring size and growth varied over runs, but it was not significant (table A4 in the online edition of the *American Naturalist*).

Table 1: ANOVA for differences in offspring size for *Watersipora subtorquata* among different regions, sites, and months

Source	df	MS	F	P	% variance
Region	1	.093	9.25	**.038 (.0214)**	25.6
Month	3	.017	1.55	.365 (.5)	< 1
Region × month	3	.011	.61	.632 (.096)	5.4
Site (region)	2	.001	.06	.946 (.5)	0
Month × site (region)	6	.018	4.50	**<.001 (.0001)**	2.5
Error	837	.004			66

Note: Significant *P* values shown in bold. We repeated our analyses using a restricted maximum likelihood (REML), log-likelihood approach and present the results of these analyses in parentheses. The percentage variance explained by each term is calculated from REML output. MS = mean square.

Table 2: ANOVA for differences in offspring size for *Watersipora subtorquata* within different regions, sites, and experimental runs

Source	df	MS	F	P	% variance
Melbourne:					
Run	9	.057	12.29	<.001 (.024)	47
Site	1	.006	1.36	.243 (.5)	<1
Site × run	9	.011	2.32	**.013 (.011)**	2
Error	986	.005			50
Sydney:					
Run	2	.0014	.17	.847 (.5)	0
Site	1	.0032	.41	.587 (.327)	3.5
Site × run	2	.0078	6.56	**.002 (.0326)**	7
Error	132	.0012			89.5

Note: Significant *P* values shown in bold. We repeated our analyses using a restricted maximum likelihood (REML), log-likelihood approach and present the results of these analyses in parentheses. The percentage variance explained by each term is calculated from REML output. MS = mean square.

Predicted Optimal Offspring Size

The optimal offspring size predicted from the offspring size-performance relationship closely matched the observed offspring sizes in the wild. The steeper relationship between offspring size and performance in Melbourne generated predictions of larger offspring size there than in Sydney; these predictions matched our observations of offspring size (fig. 4; table A5 in the online edition of the *American Naturalist*). Interestingly, the range of offspring sizes that were close (within 3% of the optimum) to the predicted optimal was greater (range: 0.193 mm^2) in Melbourne than in Sydney (range: 0.033 mm^2). In other words, the maternal fitness peak in Sydney was sharper than the peak in Melbourne, meaning that only a narrow band of offspring sizes came close to maximizing maternal fitness in Sydney. The difference in ranges matches the observed range of offspring size in Melbourne relative to Sydney (fig. 4). At a finer scale across sites and seasons, the predicted optimal offspring sizes and observed offspring size were correlated ($R^2 = 0.89$, $n = 8$, $P < .001$; fig. 5; table A5). Our model also predicted the strength and direction of change in offspring size at each site between summer and winter ($R^2 = 0.91$, $n = 4$, $P = .045$). In three sites, offspring size was predicted to increase in summer, and in one site, it was predicted to decrease in summer (fig. A3). Interestingly, across both finer scales, the range of predicted offspring sizes was greater than the range of observed offspring sizes.

Discussion

The size of *Watersipora subtorquata* offspring varied considerably across the scales that we examined, from differences among Melbourne and Sydney to differences among individual settlers. While offspring size varied significantly at higher spatial and temporal scales, most of the variation appeared to occur at lower spatial scales, within individual larval settlement events and settlement plates. It should be noted that some of the variation at the lowest scale could represent measurement error and other unaccounted sources, but overall, our observations suggest that the size of settlers on any one settlement plate is highly variable. There is a long tradition of examining geographical patterns in offspring size in marine invertebrates (Thorson 1950), and accordingly, large-scale patterns of offspring size have been a major focus of a number of studies (Barnes and Barnes 1965; Etter 1989; Dugan et al. 1991; Bridges 1993; Kokita 2003). It appears that variation at smaller scales is also substantial, and further work at these scales is warranted.

Geographic Variation in Offspring Size

Despite the surprisingly high variation in offspring size within sites and times in *W. subtorquata*, there were persistent differences in the average size of offspring between Sydney and Melbourne. Offspring in Melbourne were, on average, 40% larger than offspring in Sydney over the 3
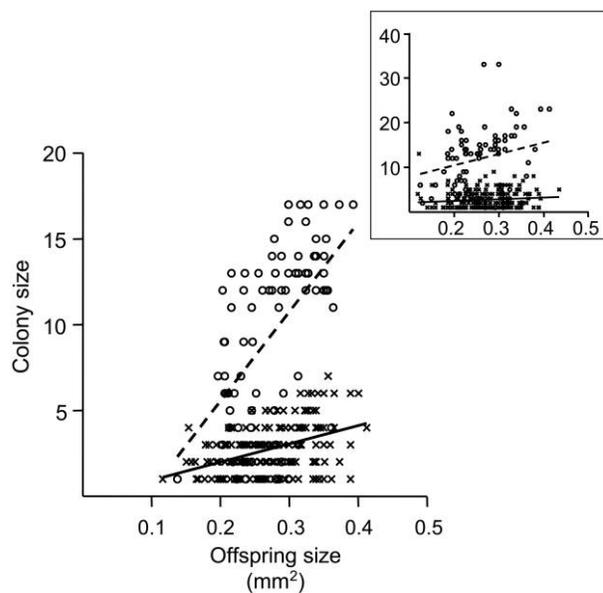

Figure 3: Relationship between *Watersipora subtorquata* offspring size and subsequent colony size (number of zooids) after 2 weeks in the field for settlers in the Melbourne region; data were pooled across experimental runs. Each point represents a single colony, and the circles and dashed lines represent offspring that settled during the summer months (March, November, December). The crosses and solid lines represent offspring that settled in the winter months (April, May, June, July). The larger panel shows the relationship between size and performance in Williamstown; the inset shows St Kilda.

Table 3: ANCOVA examining the effects of offspring size, experimental run, and site on subsequent *Watersipora subtorquata* colony size within the Melbourne region

Source	df	MS	F	P
Offspring size	1	307	26.671	**<.001**
Run	9	24	2.166	**.023**
Site	1	65	5.688	**.017**
Run × site	9	19	1.721	.081
Run × offspring size	9	63	5.542	**<.001**
Site × offspring size	1	18	1.584	.209
Run × site × offspring size	9	23	2.002	**.037**
Error	698	11		

Note: Significant *P* values shown in bold. Note that the three-way interaction suggests that the main effects tests should be interpreted with caution. MS = mean square.

months in which experiments overlapped in the two regions. In Melbourne, increased offspring size carried a greater survival and growth benefit compared with Sydney, where survival was higher overall (although still related to offspring size), but growth was generally unrelated to offspring size. In this regard, Melbourne can be regarded as a "harsher" environment in that survival was much lower there than in Sydney. The source of increase mortality in Melbourne is unknown, and an interesting next step will be to determine whether differences in predation are driving these effects. Our simple optimality model predicted a difference in optimal offspring among the two regions. Our findings are similar to those of a number of other studies on a range of taxa that found that the benefits of increasing offspring size are greater in harsher environments (Fox and Mousseau 1996; Einum and Fleming 1999; Einum 2003; Heath et al. 2003; Marshall et al. 2006; but for the opposite pattern, see Moran and Emlet 2001; Marshall et al. 2003). Interestingly, *W. subtorquata* is thought to have been a relatively recent introduction to Australian waters (a few decades; Mackie et al. 2006), but clearly, some form of change with respect to offspring size has already occurred. Whether the difference in offspring size between regions represents a genetic change or adaptive phenotype plasticity (e.g., Allen et al. 2008) remains unclear, but given the plastic nature of offspring size in marine invertebrates (reviewed in Marshall and Keough 2007), we suspect the latter.

The latitudinal variation in offspring size in *W. subtorquata* is similar to that observed among and within a wide range of species (Thorson 1950; Lardies and Castilla 2001; Lardies and Wehrtmann 2001; Moles et al. 2004), but what ecological/environmental factors drive this pattern in *W. subtorquata*? Temperature has been implicated as an important factor in several offspring size studies (Fischer et al. 2003), and water temperatures are higher in Sydney

than in Melbourne. However, if temperature were the main factor, then we would expect similar differences among seasons; if anything, larvae were smaller in winter than in summer, which suggests that temperature is not driving the latitudinal pattern (although other studies suggest that below a certain temperature, offspring sizes may actually decrease; Van der Have and De Jong 1996). Intraspecific competition can alter selection on offspring size (Marshall et al. 2006), but there were no consistent differences in the density of settlers among the two regions (table A1), and within regions, settler density was not related to settler size (table A1). However, one consistent effect that may be driving the observed differences is the relationship between offspring size and postmetamorphic growth; in Melbourne, the relationship tended to be consistently steeper, increasing the benefits of producing larger offspring. A similar pattern occurred within Melbourne across seasons; during the summer when offspring sizes were larger generally, there was a steeper relationship between offspring size and growth.

At the finer scale of sites and seasons, the offspring size that was predicted to maximize maternal fitness was correlated with observed offspring sizes among the different sites and seasons. It should be noted that much of the differences in sizes may be due to variation among the regions, but it is interesting to note that within sites, our model successfully predicted the strength and direction of change in offspring size across seasons (fig. A2). Our results suggest that, in *W. subtorquata* at least, seasonal and interpopulation differences in offspring size approximately track the changes in the relationship between offspring size and performance. For example, in times or places where there is a steeper relationship between offspring size and performance (and the production of larger offspring should be favored), the average size of offspring was greater than in times or places where there was a shallower relationship between offspring size and performance. Our

Table 4: ANCOVA examining the effects of offspring size, experimental run, and site on subsequent *Watersipora subtorquata* colony size within the Sydney region for the three experimental runs where settlement occurred at both sites

Source	df	MS	F	P
Offspring size	1	1.9	.019	.891
Site	1	492.6	4.881	**.029**
Run	2	4,678.8	46.358	**<.001**
Site × run	2	72.9	.722	.488
Error	111	100.9		

Note: Significant *P* values shown in bold. Note that the model is reduced after removing nonsignificant interactions between the covariate (offspring size) and categorical factors (see text for details). MS = mean square.
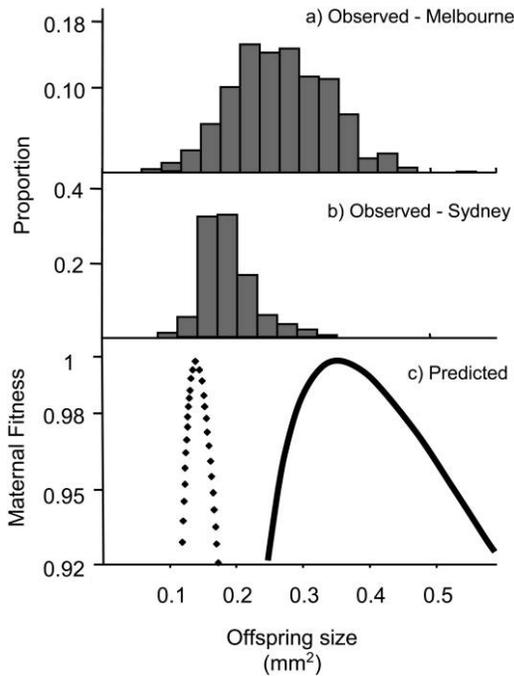
Figure 4: Size distribution of *Watersipora subtorquata* offspring naturally settling in the field in Melbourne (*a*) and Sydney (*b*). *c*, Predicted optimal offspring sizes for both regions based on optimality modeling (see text for details) and the range of offspring sizes that are within 8% of maximum maternal fitness. The broken line (*diamonds*) represents the predicted fitness returns for particular offspring sizes in Sydney, and the solid line represents the predicted fitness returns for particular offspring sizes in Melbourne.

model was successful in its qualitative predictions of offspring size, but quantitatively, the smallest and largest sizes that were predicted were more extreme than those that were observed. This highlights a problem with using natural variation in offspring sizes to infer the relationship between offspring size and performance: offspring sizes that are nonviable are unrepresented, and therefore, estimates of performance are unreliable outside of the normal range. Thus, the actual predicted values themselves are not accurate simply because the outer limits of offspring size are constrained by selection pressures that we could not estimate. Such an issue is avoided by manipulating offspring sizes beyond natural ranges and may be a more useful approach for better estimating the extremes of offspring size (e.g., Sinervo et al. 1992).

We measured the effects of offspring size on postmetamorphic performance only, but those differences in performance track the observed population-level differences in offspring size. While such a correlation provides only initial evidence, it does suggest that postmetamorphic performance is an important life-history stage with regard to

selection on offspring size (although it could be that other factors correlated with postmetamorphic performance are also influencing optimal offspring size, e.g., adult longevity). Traditional models of offspring size evolution based on planktotrophs focus on the pelagic stage (Vance 1973*a*, 1973*b*; Christiansen and Fenchel 1979; Levitan 1993; Podolsky and Strathmann 1996; McEdward 1997; Podolsky 2001). Given the strong effects of offspring size on postmetamorphic performance observed for other marine invertebrates with no pelagic feeding stage (Palmer 1990; Moran and Emlet 2001; Marshall and Keough 2003*a*; Marshall et al. 2003, 2006), we suggest that more generally, offspring size evolution in these species may be driven by postmetamorphic effects.

The larvae that settled naturally during any particular settlement event could have come from several different mothers. Thus, the high daily levels of variation in offspring size could have come from variation either among broods or within broods. Our laboratory data show that offspring size is highly variable both within and among broods, with approximately equal levels of variation at these scales. It is perhaps unsurprising that variation occurs both within and among broods; variation is inevitable in any biological system. Alternatively, both levels of variation could be a response to environmental heterogeneity. Recent studies have shown that the size of offspring that
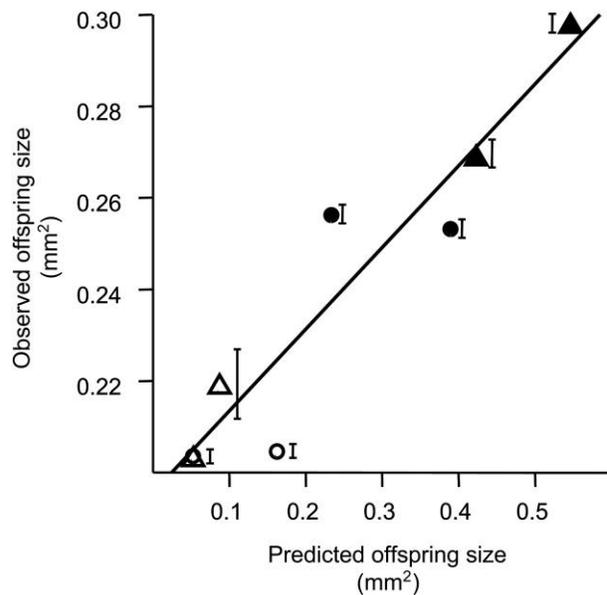

Figure 5: Predicted optimal offspring sizes and observed offspring sizes for *Watersipora subtorquata* in the field across four sites and two seasons. *Solid symbols* = sites in Melbourne, *open symbols* = sites in Sydney, *triangles* = summer sizes, and *circles* = winter sizes. Bars represent standard errors of the mean size at each site-season combination.

maximizes maternal fitness in marine invertebrates is highly variable over very small spatial scales (meters) (Moran and Emlet 2001; Marshall et al. 2006). If *W. subtorquata* colonies exhibit adaptive phenotypic plasticity with regard to offspring size and produce offspring that are of optimal size for local conditions (e.g., Fox et al. 1999), then plasticity could account for the high levels of variation among colonies, but this remains untested. With regards to within-brood variation, such variation may also represent an adaptive strategy, but the evidence for such a strategy is weaker. If mothers cannot predict the exact habitat that their offspring will colonize, and therefore the optimal offspring size is unknown, then producing offspring that are not of uniform size (i.e., bet hedging) may be favored (Capinera 1979; Lips 2001). In our study, the relationship between offspring size and performance varied over time, suggesting that the optimal offspring size (the target of selection) is dynamic, and therefore, bet hedging might be expected to be favored. We suggest that future studies on marine invertebrates examine the relationship between environmental predictability and within-brood variation (e.g., Einum and Fleming 2004) in offspring size in detail, because there is no reason to expect that selection will act only on mean offspring size—it may also act on variance in offspring size within broods.

Acknowledgments

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