OFFSPRING SIZE EFFECTS MEDIATE COMPETITIVE INTERACTIONS IN A COLONIAL MARINE INVERTEBRATE

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Abstract. Over the past 30 years, numerous attempts to understand the relationship between offspring size and fitness have been made, and it has become clear that this critical relationship is strongly affected by environmental heterogeneity. For marine invertebrates, there has been a long-standing interest in the evolution of offspring size, but there have been very few empirical and theoretical examinations of post-metamorphic offspring size effects, and almost none have considered the effect of environmental heterogeneity on the offspring size/fitness relationship. We investigated the post-metamorphic effects of offspring size in the field for the colonial marine invertebrate *Botrylloides violaceus*. We also examined how the relationship between offspring size and performance was affected by three different types of intraspecific competition. We found strong and persistent effects of offspring size on survival and growth, but these effects depended on the level and type of intraspecific competition. Generally, competition strengthened the advantages of increasing maternal investment. Interestingly, we found that offspring size determined the outcome of competitive interaction: juveniles that had more maternal investment were more likely to encroach on another juvenile's territory. This suggests that mothers have the previously unrecognized potential to influence the outcome of competitive interactions in benthic marine invertebrates. We created a simple optimality model, which utilized the data generated from our field experiments, and found that increasing intraspecific competition resulted in an increase in predicted optimal size. Our results suggest that the relationship between offspring size and fitness is highly variable in the marine environment and strongly dependent on the density of conspecifics.

Key words: Botrylloides violaceus; *competition; egg size; maternal effects; progeny size.*

INTRODUCTION

The study of offspring size variation is a fundamental component of evolutionary ecology (Stearns 1992). Because the capacity of mothers to produce offspring is finite, they can either produce many small offspring or fewer, larger offspring (Lack 1947, Vance 1973*a,* Smith and Fretwell 1974). A central tenet of life-history theory therefore is that larger offspring have higher fitness than smaller offspring and this differential fitness offsets the difference in fecundity (Stearns 1992). Indeed larger offspring have higher fitness in a wide range of taxa (Stanton 1984, Williams 1994, Bernado 1996), but our understanding of offspring size and fitness is far from complete. Initial attempts at modeling ''optimal'' offspring size assumed a constant relationship between offspring size and fitness (Vance 1973*a,* Smith and Fretwell 1974). We now recognize that the relationship between offspring size and fitness is highly variable, and it is unlikely that any single offspring size will be

optimal (Fox et al. 1997, Einum and Fleming 2002). The relationship between offspring size and fitness may be strong or weak depending on environmental conditions, and for some organisms this can dramatically alter selection on offspring size (Kaplan 1992, Fox and Mousseau 1996, Einum 2003). For example, Fox (2000) found that the intensity of selection on egg size in the seed beetle, *Stator limbatus*, strongly depended on the resistance of seed coats to the beetle larvae. Knowledge of the interaction between offspring size and environmental variation is therefore crucial to our understanding of the evolution of offspring size. For marine organisms, the effects of environmental heterogeneity on the relationship between offspring size and fitness have received little attention (but see Moran and Emlet 2001).

The paucity of studies examining the effects of environmental variation on the relationship between offspring size and fitness in marine organisms is remarkable given the extremely dynamic nature of the marine environment. For marine organisms, the environment into which offspring are born can be extremely variable at remarkably small scales. Temperature, wave exposure, food availability, and the abundance of predators

can be highly variable over just a few meters, and each of these factors is likely to strongly affect the relationship between offspring size and fitness (Rivest 1983, Etter 1989, Moran and Emlet 2001). One of the most important and common ways in which the environment can vary for marine organisms is the density of conspecifics in the habitat in which they settle (Underwood and Keough 2001). Densities of newly settled larvae can vary enormously in space and time (reviewed in Gosselin and Qian 1997, Hunt and Sheibling 1997, Underwood and Keough 2001) and can strongly affect subsequent survival and growth in benthic invertebrates (Hurlbut 1991, Dalby 1995). Furthermore the availability of free space and the proximity of newly settled larvae to established adults can be highly variable (Connell 1961, Keough 1983, Keough 1984*a, b*) and can also affect subsequent survival and growth (Connolly and Roughgarden 1999). Thus, variation in the abundance of conspecifc settlers and adults is likely to strongly affect the relationship between offspring size and fitness but has never been examined in marine invertebrates. In the analogous situation of the effect of seed size and competition on subsequent performance in plants, Stanton (1984) concluded that incorporating the effects of competition was crucial for any study of offspring size to be realistic. Given the prevalence of competition in marine assemblages it is surprising that there are so few studies examining competition's effects on the offspring size/fitness relationship (see Marshall and Keough 2003*a* for exception).

There have been numerous attempts to mathematically describe selection on offspring size in marine invertebrates (Vance 1973*a, b*, Havenhand 1993, Levitan 1993, 2000, Podolsky and Strathmann 1996). However, the majority of these models focus on pre-metamorphic events (but see Havenhand 1993) and fail to consider the effects of offspring size on post-metamorphic survival and growth, despite earlier work showing offspring size may strongly affect these parameters (Bagenal 1969, Rivest 1983). Because offspring size can strongly affect post-metamorphic survival, growth, and reproduction (Bervan and Chadra 1988, Williams 1994, Einum and Fleming 1999, Gimenez and Anger 2001, Moran and Emlet 2001, Marshall et al. 2003*a*), models of offspring size for marine organisms should reflect these effects. One can imagine a situation where a model predicts an optimal offspring size that maximizes the number of larvae achieving metamorphosis, but without considering post-metamorphic survival it may predict an offspring size that will result in no juveniles surviving. Models incorporating post-metamorphic effects are necessary if we hope to understand the evolution of offspring size in marine invertebrates, which exhibit an impressive level of variation in developmental mode and offspring size.

Here, we examine how maternal investment and competition affect post-metamorphic survival and growth in the field for the colonial ascidian *Botrylloides violaceus*. We first determined the relationships between offspring size and survival and between offspring size and growth, then we examined how competition affected these relationships. We examined the effects of three forms of competition, each of which are likely to occur in the field: noncontact competition between settlers at high density (Dalby 1995, Marshall and Keough 2003*a*), contact competition between newly settled larvae, and competition between newly settled larvae and established, adult colonies (see Appendix A for our definitions of noncontact and contact competition). Using the results of these experiments, we then created a simple optimality model and compared predicted optimal offspring size under the different competitive regimes in this study.

MATERIALS AND METHODS

Study species and study site

Botrylloides violaceus is an encrusting colonial ascidian that grows by asexual budding and spreads out across hard substrates. Its non-feeding larvae spend only a short time in the plankton before attaching to a surface, quickly metamorphosing, and beginning to feed (<24 h). Here we define "settlement" as the attachment to a substrate and initiation of metamorphosis. All collections of reproductive adults and experiments were done in the inner boat basin of Charleston, Oregon, USA (43°20.730′ N, 124°19.610′ W). This boat basin is sheltered from the prevailing weather by a rocky breakwater. *Botrylloides violaceus* is one of the most abundant fouling organisms coating the floating docks and pier pilings. The sea-surface temperature during the study period (July–August 2004) was around 10°C.

Collection of larvae

Reproductive colonies were collected from the undersides of floating docks and transported back to the laboratory in buckets. The colonies were maintained in aquaria with recirculating seawater (10°C) in complete darkness for 1–3 days in which time they received no supplemental food. To obtain larvae, we exposed the colonies ($n = \sim30$) to bright light and gently tore them into small (~8 cm^2) pieces; both of these stimuli caused colonies to release large numbers of larvae. These larvae were collected with glass pipettes and quickly checked to insure that they were fully developed.

General experimental methods

Larval size was measured as the head length of the tadpole larva according to the methods described in Marshall and Keough (2003*b*). To measure settler size, we placed each freshly collected larva into its own pre-roughened petri dish (90 mm diameter) filled with seawater. We placed the dishes of larvae into an incubator at 10°C and allowed them three hours to settle. Un-

settled larvae were discarded. We moved petri dishes containing settled larvae to a recirculating seawater system at 10°C and allowed the settlers to metamorphose for 24 hours, after which time the settlers were photographed at 40× with a digital camera attached to the microscope. The images of settlers were analyzed using Optimas version 6 (Meyer Instruments, Houston, Texas, USA) and settler size was estimated from the area of the branchial basket (the pigmented area of the settler; see Marshall et al. 2003b for details).

To deploy measured settlers into the field we drilled a small (3 mm diameter) hole in the center of each petri dish, placed the dishes into finger bowls filled with seawater, and transported them to the field site (a five-minute journey). We fixed the petri dishes containing settlers to a PVC backing plate (~100 × 50 cm) with stainless steel bolts inserted through the holes. The backing plate was then suspended face down (to reduce the effects of light and sedimentation) at a depth of 1 m below the water surface. Each experimental run was deployed on a single backing plate and petri dishes were treated as replicates. This is appropriate as the treatments were applied at the level of dish, and we detected no run × treatment interactions (see *Results*).

To measure the colonies, we removed the petri dishes from the backing plates and returned the colonies to the laboratory in glass finger bowls filled with seawater. The colonies were then photographed under a dissecting microscope (variable magnification depending on the size of colonies), and colony size was estimated as colony area from these images.

Variation in the size of field settlers

To determine if our laboratory collected settlers were of representative size, we collected settlers from the field. We deployed 10 pre-roughened, black Perspex settlement plates (10 × 10 × 0.8 cm) face down on backing plates as described previously. We deployed our plates at ~19:00 in the evening. *Botrylloides violaceus* larvae are released between 8:00 and 12:00 at Charleston and so no settlement occurred during the night (D. J. Marshall and C. N. Cook, *personal observation*). At ~13:00 the next day we retrieved the settlement plates, brought them back to the laboratory, and placed them in aquaria (10°C) for ~20 hours. After this time, we measured the size of the settlers as described previously. We repeated this 10 times during the study period.

Effect of settler size on post-metamorphic survival and growth

To examine the effects of settler size on post-metamorphic survival and growth in the field, we collected larvae, allowed them to settle in petri dishes (one settler per petri dish), and measured the resultant settlers as described previously. Settler size was highly variable and we took care to collect a range of larval sizes from each colony. Every week for the first three weeks in the field, we returned the petri dishes to the laboratory and assessed colony survival and growth. The entire process took ~90 minutes each week so colonies were absent from the field site for only a short period. We did not census the colonies after four weeks in the field, but we did census the colonies again after five weeks in the field. We deployed two experimental runs to examine the effect of settler size on post-metamorphic survival and growth, one was deployed on 4 July (*n* = 41), the other was deployed (*n* = 19) on a separate backing plate on 5 July.

The effect of settler size on colony survival after one week and five weeks in the field was tested using logistic ANCOVA where settler size was a covariate and run was a random factor. For all analyses, Wald tests were used with one degree of freedom. After finding the interaction for run × settler size was not significant, it was removed and the analysis was run again. Effect of settler size on colony size was tested using repeated-measures ANCOVA where settler size was a covariate, run was a random factor, and time was a fixed factor. We first included run × settler size in the analyses, however given this interaction was not significant and of no biological interest, we removed it from the model and then re-ran the analyses.

Effect of settler size on initial budding rate

We collected laboratory-released settlers (*n* = 37 from eight colonies), measured, and deployed them into the field as described previously. After 1 day in the field (48 hours after metamorphosis), we photographed the developing colonies and counted the number of newly budded zooids (blastozooids) before returning the petri dishes to the field. After three days in the field (96 hours after metamorphosis), the petri dishes were retrieved and again, the number of blastozooids was counted.

Effect of settler size on growth and colony form at high and low settlement densities (noncontact competition)

After collection, we assigned larvae haphazardly into either the "low" density group or the "high" density group. The low density group had 2–4 settlers per petri dish (mean: 3.5 settlers per dish) and the high density group had 9–13 settlers per petri dish (mean: 11 settlers per dish). These densities are representative of the range of values observed in the field. Care was taken to ensure that no two larvae from the same colony were on the same petri dish. After metamorphosis and measurement, the settlers were deployed (*n* = 36 settlers across eight petri dishes) into the field on 12 July. We then monitored survival and growth every week for three weeks. We kept track of the individuals by maintaining gray-lead circles around each individual.

We analyzed the effect of settler size and density on subsequent colony growth with a repeated-measures

ANCOVA where settler size was a covariate and density was a fixed factor.

There appeared to be a relationship between the density of colonies on a petri dish and colony growth form, so in a subsequent experiment we specifically examined how density affected the appearance of colonies. We used two density treatments: high (>5 settlers per petri dish) and low (<5 settlers per petri dish). The experiment was deployed into the field on 30 July with 16 petri dishes and a total of 73 settlers (eight replicate dishes of each treatment). After one week in the field, we measured the size of colonies, and the number and size of zooids within each colony (measured according to method described previously). We analyzed the effect of density on two response variables, (1) the ratio of zooids to colony area, and (2) zooid size, with a nested ANOVA where density was a fixed factor and petri dish was a nested, random factor.

Effect of settler size on contact interactions of settlers

To examine contact competition between new settlers, we settled individual larvae adjacent to each other (where their sizes varied) and determined which of the two colonies grew into each other's "territory" after two weeks in the field. To settle larvae adjacent to each other, we collected larvae as described before and then placed two randomly chosen larvae (from different colonies) onto a dry petri dish in a single drop of seawater. This prevented the larvae from settling too far away from each other (most of the settlers were within 1 mm of each other). The settling larvae were then allowed to attach and develop as described previously before being measured. Once the settlers had been measured, the settlers were rephotographed under a dissecting microscope with a small pencil mark made adjacent to them as a landmark. The petri dishes ($n = 15$ dishes/ interacting pairs) were then deployed into the field on 27 July and after several days, the developing pairs of colonies were in contact with each other. A digital line was then drawn on the image, exactly in the middle of the two settlers. After two weeks the petri dishes were retrieved and photographed again, taking care to include the pencil landmark. The digital line from the initial image was then placed onto the image of the colonies after two weeks in the field, and we used this line to determine which colony (if any) had crossed the line into the other's territory (14 out of 15 pairs showed one colony encroached upon another). The colony that encroached upon the other's territory was classed as the aggressor in each case.

We analyzed the results of this experiment with a logistic regression where the difference in the sizes of the two settlers was the predictor variable and aggression or nonaggression was the response variable. To calculate the difference in the sizes of the two settlers, one settler was selected at random and designated the focal settler; the difference in size between this settler and the other was then calculated and we determined if the focal settler was the aggressor.

Effect of settler size on post-metamorphic survival and growth in the presence of established colonies

We examined the effects of settler size on survival and growth of colonies in the presence and absence of established conspecifics. To produce established competitors, we settled larvae onto petri dishes (one larva per dish) five weeks earlier, placed them into the field, and allowed them to grow for five weeks, after which we returned them to the laboratory. We then collected larvae from other colonies as described previously and haphazardly allocated them into two groups. In the "no competition" group ($n = 15$), larvae were allowed to settle on petri dishes at a density of one larva per dish. In the "competition" group ($n = 22$), larvae were settled directly adjacent to the established colonies. To do this we used small (10 mm diameter) polyethylene tubes that sat directly next to an established colony; the tube contained a small volume (~1 mL) of seawater. We placed five larvae in each tube and allowed one hour for settlement. Any larvae that had failed to settle within one hour, or settled on the tube, were discarded and if more than one larva had settled, the additional settlers were removed at random leaving only one settler. We measured the settlers of both groups 24 hours after settlement and then deployed them into the field on 10 August. After 10 days, we examined survival and growth of the settlers in both treatments.

In the no competition group there was almost no mortality and so we used logistic regression to examine whether mortality was related to settler size for the competition group only. We analyzed the effect of settler size, and competition with established competitors, on colony growth with an ANCOVA where settler size was a covariate and presence/absence of established colonies was a categorical factor. In all instances, the data were not badly skewed and we analyzed raw data.

RESULTS

Variation in the size of settlers in the field

We found that larval size and settler size were highly correlated (linear regression, $R^2 = 0.78$, $P < 0.001$, $n = 17$). The size of settlers that naturally settled in the field was extremely variable (mean = 0.0387 cm^2, CV = 45% (0.01–0.08 cm^2), $n = 31$ settlers over 10 samples) and encompassed the size range of settlers used throughout our experiments.

Effect of settler size on initial budding rate

Forty-eight hours after metamorphosis, settler size was a good predictor of initial budding rate with larger settlers producing more blastozooids than smaller settlers ($R^2 = 0.301$, $P < 0.001$, $n = 37$; equation, no. buds = 93.6 [settler size] + 0.51). Though more variable 96 hours after metamorphosis, larger settlers were

TABLE 1. Repeated-measures ANCOVA examining the effects of settler size and experimental run on subsequent colony size for *Botrylloides violaceus* in the field.

Source	df	MS	F	P
Between subjects				
Settler size	1	5.695	12.786	0.001
Experimental run	1	1.737	3.900	0.055
MS$_{residual}$	42	0.445		
Within subjects				
Time	3	0.158	1.096	0.337†
Time × settler size	3	0.787	5.464	0.007†
Time × run	3	0.766	5.320	0.007†
MS$_{residual}$	126	0.144		

Note: The model was reduced after testing for and finding a nonsignificant interaction between experimental run and settler size.

† Greenhouse-Geisser adjusted *P* values reported ($\varepsilon = 0.633$).

still more likely to have more blastozooids ($R^2 = 0.136$, $P = 0.018$, $n = 37$; equation, no. buds = 67.3 [settler size] + 1.5).

Effect of settler size on post-metamorphic survival and growth

Mortality was very low at the start of the experimental runs with 95% survival in the first week and 81% survival after five weeks. Initial survival of colonies after one week was independent of original settler size (settler size, $\chi^2 = 1.317$, $P = 0.251$; run, $\chi^2 = 0.002$, $P = 0.964$), but survival after five weeks in the field was size dependent, with colonies from larger settlers more likely to survive than colonies from smaller settlers (settler size, $\chi^2 = 5.732$, $P = 0.017$; run, $\chi^2 = 0.001$, $P = 0.979$).

Settler size strongly affected colony size with larger settlers becoming larger colonies. The effect of settler size on colony size persisted for five weeks in the field but became more variable through time (Table 1, Fig. 1). In run one, settler size explained 46% of variation in colony size after one week in the field but explained only 22% of the variation after five weeks in the field (values calculated from R^2 of linear regression). In run two, settler size explained 67% and 14% of variation in colony size after one week and five weeks in the field, respectively.

Effect of settler size on post-metamorphic survival and growth at different densities of settlers (noncontact competition)

Mortality was low with only a single colony dying during three weeks in the field so mortality was not analyzed. We found a strong interaction between settler size and the density of settlers on subsequent colony growth (Table 2). The difference in growth rates between colonies from large and small settlers was enhanced at high densities of settlers (i.e., the differences in growth rates between colonies from large and small settlers were increased at higher densities; Fig. 2a). The

interaction between settler size and density persisted into the third week; however it should be noted that this interaction appeared to be heavily influenced by a single colony in the low density treatment (Fig. 2b).

Effect of settler density on growth form (noncontact competition)

Settler density strongly affected colony growth form, with colonies at higher densities having zooids 20% smaller than colonies at lower densities (0.028 ± 0.001 cm^2 vs. 0.035 ± 0.002 cm^2, Table 3). This difference appeared to be due to the fact that in the high density treatment, each large oozooid had many smaller zooids budding off, reducing the overall mean zooid size. Settler density also affected the number of zooids per unit of colony area (high density of 7.24 zooids/cm^2 vs. low density of 9.8 zooids/cm^2, Table 3). Generally, colonies at higher densities appeared more "spread out" with
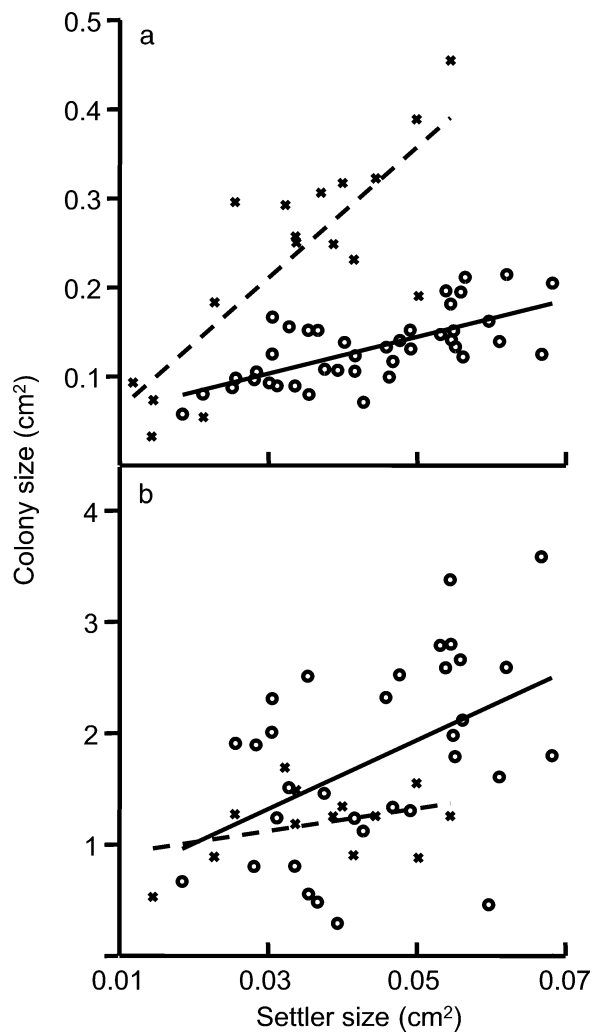

FIG. 1. Effect of settler size on subsequent colony size after (a) one week and (b) five weeks in the field for *Botrylloides violaceus*. Each point represents a single colony; run 1 is represented by circles and run 2 by crosses.

TABLE 2. Repeated-measures ANCOVA examining the effects of settler size and settler density on subsequent colony size for *Botrylloides aurantius* in the field.

Source	df	MS	F	P
Between subjects				
Settler size	1	1.023	23.19	<0.001
Settlement density	1	0.227	5.15	0.031
Settler size × density	1	0.232	5.249	0.030
$MS_{residual}$	27	0.044		
Within subjects				
Time	2	0.001	0.047	0.852†
Time × settler size	2	0.112	5.85	0.018†
Time × settler density	2	0.060	3.11	0.082†
Settler size × density × time	2	0.063	3.28	0.074†
$MS_{residual}$	54	0.019		

† Greenhouse-Geisser adjusted *P* values reported ($\varepsilon = 0.577$).
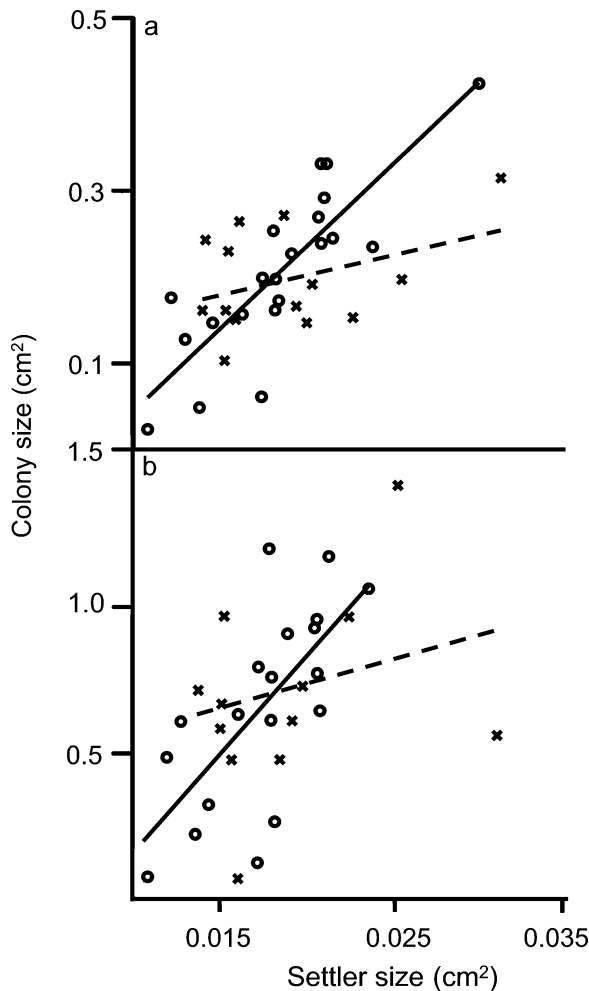

FIG. 2. Effect of settler size and noncontact competition on colony size (a) after one week and (b) three weeks in the field for *Botrylloides violaceus*. Both panels show the effect of settler size and two settlement densities: crosses and dashed line indicate low settlement densities; circles and solid line indicate high settlement densities. Each point represents a single settled colony.

larger colony margins that did not contain zooids, as was reflected by the ratio of colony area to zooid number.

Effect of settler size on contact interactions between settlers

The difference in the size of directly competing settlers strongly influenced the outcome of the competitive interaction, with larger settlers being much more likely than smaller settlers to be aggressors ($\chi^2 = 17.944$, df $= 1$, $P < 0.001$, Fig. 3). Colonies originating from smaller settlers appeared to show some directed growth (in the orientation of their zooids and newly budded zooids) away from the colony originating from the larger settler. Furthermore, colonies from smaller settlers generally appeared to be thinner (looking on the colonies from above) than colonies from larger settlers.

Effect of settler size on post-metamorphic survival and growth in the presence of established colonies

The presence of established colonies had a strong effect on settler survival, 63% of settlers survived in the presence of established competitors whereas 93% survived in the absence of established competitors. The main source of mortality in the competition group appeared to be overgrowth by established colonies. Within the competition treatment, the survival of settlers was strongly dependent on original settler size, with

TABLE 3. Effect of settler density on zooid to colony area ratio and mean zooid number for *Botrylloides violaceus* after one week in the field.

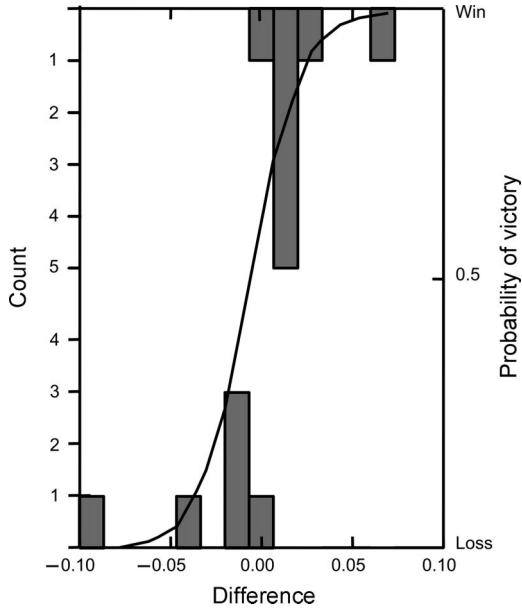| Source	df	MS	F	P
Zooid : colony area				
Density	1	0.017	16.903	0.001
Plate (density)	14	0.001	0.386	0.979
$MS_{residual}$	56	0.003		
Zooid size				
Density	1	60.11	19.67	0.001
Plate (density)	14	3.05	0.472	0.943
$MS_{residual}$	56	6.5		

FIG. 3. Effect of the difference in settler sizes on outcome of competitive interactions for *Botrylloides violaceus*. The bottom histogram represents the distribution of differences in settler size that resulted in the focal settler being acquiescent (i.e., if $x < 0$ then the focal settler was smaller; if $x > 0$, then the focal settler was larger). The top histogram represents the distribution of differences in settler size that resulted in the focal settler being an aggressor. The line represents the logistic regression line of best fit.

larger settlers having a greater chance of survival than smaller settlers ($\chi^2 = 5.25$, $P = 0.022$, Fig. 4)

The presence of established competitors also strongly affected growth, with colonies in the presence of competitors growing more slowly than colonies in the absence of competitors (Fig. 5). Overall, settler size
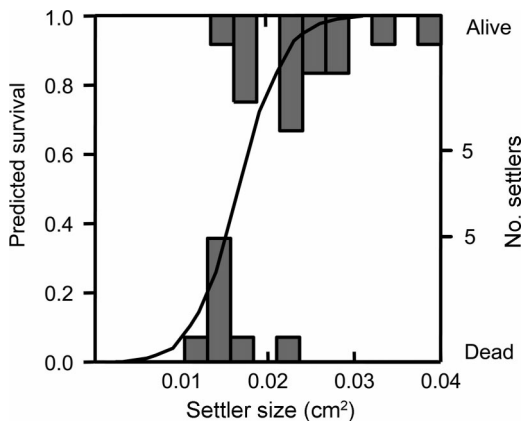

FIG. 4. Effect of settler size on predicted survival of *Botrylloides violaceus* colonies in the presence of established colonies after 10 days in the field. The bottom histogram represents the size distribution of settlers that died, and the top histogram represents the size distribution of settlers that lived. The line represents the logistic regression line of best fit.
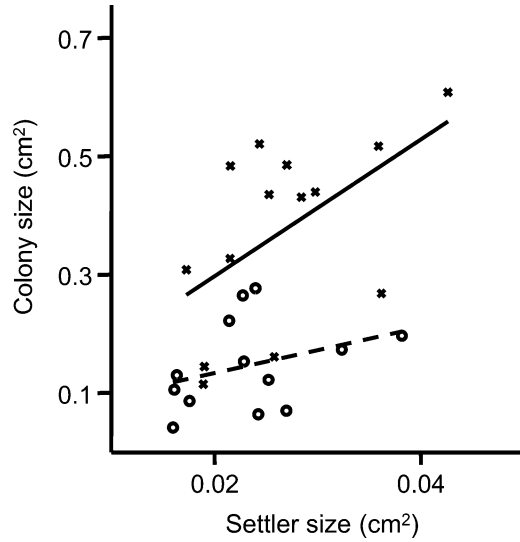

FIG. 5. Effect of settler size and the presence or absence of established colonies on the size of *Botrylloides violaceus* colonies after 10 days in the field: crosses and solid line indicate no established colonies present; circles and dashed line indicate established colonies present. Each point represents a single settled colony.

affected colony growth with larger settlers achieving a larger colony size in both the presence and absence of established competitors (Table 4).

POST-METAMORPHIC EFFECTS MODEL

We used an optimality model to examine how optimal offspring size changed under different competition regimes. The model used estimates of the effect of offspring size on survival and growth, from the results our experiments, as constants and we varied offspring size across the range of values observed in this study. The model is restricted to the effects of settler size on post-metamorphic performance; the effects of settler size on pre-metamorphic survival for this species remain unknown and for simplicity were excluded. We used settler area as our offspring size measure, so to calculate offspring volume, and therefore investment, we raised our size measure to the power of 1.1. This was done because an increase in settler area resulted in only a small change in the thickness of the settlers. As in previous models (e.g., Smith and Fretwell 1974, Levitan 1996) the number of offspring pro-

TABLE 4. Effect of settler size and presence/absence of established colony on colony size for *Botrylloides violaceus* after one week in the field.

Source	df	MS	F	P
Settler size	1	0.085	6.805	0.015
Competition	1	0.255	20.411	0.000
MS$_{residual}$	24	0.013		

Note: Model is reduced after testing for homogeneity of slopes.

duced by mothers is inversely proportional to per off-spring investment:

$$N = \frac{M}{s^{1.1}} \times p \qquad (1)$$

where N = number of settling larvae that are produced, M is the amount of resources available for reproduction (an arbitrary value kept constant throughout), s is offspring size (measured as settler area), and p is survival in the plankton (again an arbitrary value, 0.05, kept constant throughout). To predict how many settling larvae will survive through three weeks of benthic life we used the following equation:

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

where α and β are constants derived from the logistic regression of settler size vs. survival (Appendix B). To predict the size of colonies after three weeks in the field, we used the equation

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

where γ and δ are constants derived from the linear regression equations of settler size vs. colony growth after three weeks in the field (Appendix B). We then used the cumulative size of each offspring colony (which is a product of individual colony growth rate and survival) as a surrogate for maternal fitness. This is likely to be a good surrogate given that colony survival and fecundity are typically correlated with colony size in colonial invertebrates (Sebens 1987, Keough 1989, Marshall et al. 2003a). Maternal fitness, ψ, then is given by putting Eqs. 1, 2, and 3 together to produce the equation

$$\psi = N \times B \times G. \qquad (4)$$

We then plotted maternal fitness vs. settler size under three different conditions: no competition, established competitors present, and high settlement densities. In each instance, we used the appropriate regression equations to estimate the constants α, β, γ, and δ. Finally, for simplicity we constrained ψ to vary between 0 and 1; the actual values will depend on maternal resources, planktonic mortality, and so on, all of which were held constant in this model (it should be noted that maternal fitness was much lower overall under competition than in the absence of competition).

Model results

When no competition was present, the model predicted an optimal offspring size of 0.019 cm², although a wide range of sizes was close to optimal under these conditions (Fig. 6a). When settlers were in the presence of established competitors, there was an increase in optimal offspring size (0.025 cm²) and the range of sizes that were close to optimal decreased (Fig. 6b). When settler densities were higher, again increased per
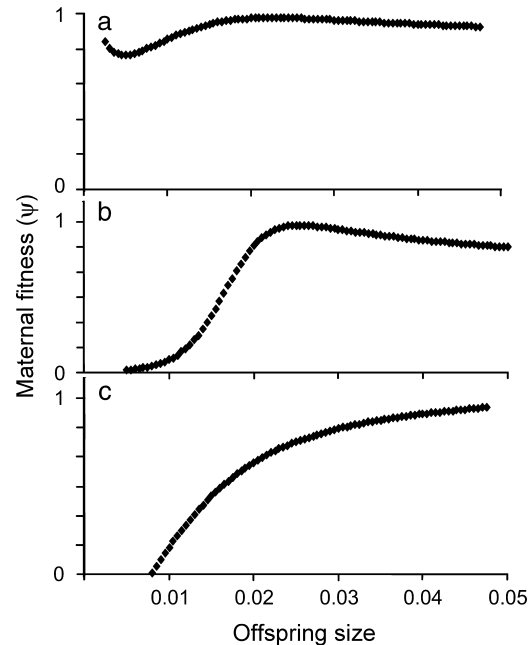

FIG. 6. Output from optimality model under three different competitive regimes: (a) no competition, (b) established competitors present, and (c) high density of settlers. Note that under intraspecific competition (c), maternal fitness is maximized when larger offspring are produced.

offspring investment resulted in higher maternal fitness but there was no optimal size under these conditions; mothers were predicted to be better off producing offspring as large as possible (Fig. 6c).

DISCUSSION

Effect of settler size in the absence of competition

The effects of settler size on subsequent colony survival in the absence of competition were weak and were only apparent after a long period in the field. Relative mortality was generally low among all of the experiments compared to other studies (Hunt and Sheibling 1997, Marshall et al. 2003a). For the longest experimental run, initial colony mortality was independent of settler size, but after five weeks in the field, we found an effect of settler size on survival. We suggest that settler size indirectly affects post-metamorphic survival because larger colonies have higher survivorship and settler size strongly affected colony growth. This contrasts with previous studies on the bryozoans *Bugula neritina* and *Watersipora subtorquata*, which show that settler size affects survival initially but subsequent survival is random with respect to settler size (Marshall et al. 2003a, Marshall and Keough 2004). This may be because metamorphosis is much more costly (relative to initial provisioning) for bryozoans than for colonial ascidians where the adult structures are already partially formed inside the larva (Bennett and Marshall 2005).

In contrast to the effects of settler size on mortality, the effects of settler size on subsequent colony size were strong and quickly apparent in all the experimental runs. In all cases, larger settlers became larger colonies. This effect of settler size on subsequent colony growth was probably due to the early effects of settler size on the production of new blastozooids from the oozooid. Larger settlers produced many more blastozooids than smaller settlers after only 48 hours in the field. This difference in initial budding rate probably gave larger settlers an advantage in initial feeding and growth, which compounded over time. The bryozoan *Watersipora subtorquata* shows similar effects of settler size on subsequent budding rate (Marshall and Keough 2004). The effects of settler size on subsequent colony size persisted for at least five weeks in *B. violaceus*, which contrasts with similar studies on *W. subtorquata* and the ascidian *Diplosoma listerianum* (Marshall and Keough 2004, 2005). For these species, the effects of settler size on colony growth were initially strong, but they disappeared as colonies were surrounded by other organisms competing for space (Marshall and Keough 2004). In the present study, natural recruitment to Petri dishes deployed in the field was not very high and the experimental colonies of *B. violaceus* were not crowded or obviously competing with other taxa. It is possible that the effects of settler size on colony growth persisted because of the availability of free space. *Botrylloides violaceus* is known to be a dominant competitor (Bullard et al. 2004), and so it would be interesting to know if the effect of settler size on colony growth persists even in the presence of other fouling species.

Effects of settler size and competition

The effects of offspring size were generally more pronounced in the presence of competitive interactions but were dependent on the type of competitive interaction. When settlers were in the presence of established colonies and presumably under more stressful conditions, settler size had strong effects on survival, but when established colonies were absent, mortality was minor. The major source of mortality appeared to be overgrowth by the established colonies. We had expected both larger and smaller settlers to be equally likely to be overgrown by the larger established colonies and were surprised that settler size had an effect. The faster growth rate of larger settlers appeared to help them avoid overgrowth from the advancing, established colonies. Thus the disadvantage of smaller settlers is magnified when established colonies are abundant.

Overall, the growth rate of new colonies in the presence of established colonies was reduced, suggesting that not only are established colonies competing for space, they are also competing for food by locally depleting the available food for developing colonies. Competition for food has been invoked as a mechanism for competition in a number of benthic marine invertebrates (Dalby 1995, Marshall and Keough 2003a). However allelopathy could also explain the reduced growth rates and has been documented in a number of different colonial marine invertebrates (Engel and Pawlik 2000). Further work is necessary to distinguish between these two hypotheses.

Contrary to our expectations, larger settlers were not more likely to overgrow smaller settlers when they were in direct contact with (or in very close proximity to) each other. Rather, smaller settlers were more likely to lose territory to the larger settler. Furthermore, it appeared that the growth of zooids from smaller settlers was oriented away from the larger settler. This directed growth away from the larger settler was similar to what we observed in the previous experiment when new settlers were positioned near established colonies. Under natural conditions, free space is likely to become limited and therefore the scope for smaller settlers to grow away from larger settlers is likely to be limited. In marine epibenthic communities, where competition for space is prevalent, competitive hierarchies exist whereby one species typically dominates another (Buss 1979, 1980, 1990, Russ 1982). However, these hierarchies can vary through time and space and ''reversals'' (i.e., where a usually subordinate species defeats a dominant species) are common (Buss 1990). While a number of factors have been suggested to affect the outcome of competitive interactions (Dunstan and Johnson 2003), few have considered the role of maternal provisioning. Colony size can affect the outcome of competitive interactions within species (Sebens 1982, Nandakumar and Tanaka 1997), and maternal provisioning (through effects on colony growth rates) may also affect the outcome of competitive interactions among species.

There was a strong interaction between settler size and density on subsequent growth rates. The effects of settler size on growth were greatly increased at higher settlement densities. In the presence of more settlers, larger settlers had even higher growth rates and smaller settlers had even lower growth rates relative to similar size settlers at low densities. Settler density also strongly affected the growth form of colonies. At higher densities, colonies spread out more, relative to the number of zooids they contained, and zooid sizes were generally smaller. This difference in growth rates may be a plastic response by colonies so that they can preempt as much space as possible before contact between colonies is made. As discussed previously, overgrowth of one colony by another of approximately the same age appears rare in this species and only when there were large differences in size/age did we observe overgrowth. So it may be that this preemption of space is an effective way of securing space before it is occupied by another colony. This suggests that colonies can detect other nearby colonies through some waterborne cue, either diminished food supply or some sort of chemical substance released by conspecifics. Pheno-

typic plasticity is common in colonial marine invertebrates (Harvell 1992, 1994), and it may be that this represents another, previously undocumented response. However, an accelerated budding rate is probably not without costs—colonies at higher densities also had smaller zooids overall and this probably means that their ability to feed is diminished (Okamura 1984, Wendt 1996). This may explain the interaction between settler size and density on growth. When colonies come from larger settlers, they are better able to sustain the higher growth rate even in the face of increased competition. In contrast, smaller settlers at higher densities are likely to have less available food and will have fewer maternally derived resources to sustain a high rate of zooid budding; therefore their growth is diminished relative to settlers at low densities.

Interestingly, in the experiment where the density of settlers varied, there was no interaction between settler size and density on subsequent survival, but there was a strong interaction on growth. The converse was true when established colonies were present: there was a strong interaction between the influence of settler size and colony presence on survival (all survived when no established colonies were present but smaller settlers died when there established colonies) but not growth. Why does one form of competition result in effects on growth but another form result in effects on survival? We suggest that settler size is affecting growth rates in both experiments but when selection for fast growth is more intense (in the presence of established colonies) the effect is manifested as an effect on survival. More generally, we would expect greater stresses to affect the relationship between offspring size and survival and milder stresses to affect the relationship between offspring size and growth. While speculative, this idea warrants further exploration.

Our simple optimality model shows that when there is no competition, a broad range of offspring sizes are close to optimal, maximizing the area occupied by offspring. This suggests that there is a balance between producing fewer, fitter offspring and more numerous, less fit offspring. Because we did not measure the energetic content of large and small larvae we cannot conclusively state that larger offspring were more energetically expensive to produce. However, we are confident that mothers face a trade-off between the size and number of offspring they can produce for several reasons. First, while some studies have shown that there is no relationship between offspring size and energetic content within marine invertebrate species (e.g., McEdward and Coulter 1987), most have shown that there is a strong relationship including species in which previous studies have found no relationship (McEdward and Chia 1991, Clarke 1993, Bingham et al. 2004). Second, irrespective of energetic costs, mothers still face a trade-off between the size and number of offspring that they can produce because of simple brood capacity constraints (Strathmann et al. 1984). We

found that several different forms of intraspecific competition increased the predicted optimal offspring size. The presence of established competitors affected optimal offspring size because smaller settlers suffer much higher mortality when established colonies are present. Surprisingly, competitors did not need to be established to affect the predicted optimal size. This is because at high settlement densities, settler size affected colony growth much more strongly than at low settlement densities. Our model confirms what our empirical data suggested, that optimal offspring size is highly dependent on the offspring environment, specifically, the presence of conspecific competitors. Interestingly, a broad range of offspring sizes are still very close to optimal size when there is no competition and generally, as long as settlers were larger than ~ 0.02 mm^2, mothers had close to optimal fitness under all conditions. We observed a remarkable level of variation in the size of larvae that settled naturally in the field, but $<10\%$ of these individuals were smaller than 0.02 mm^2. It may be that given the lack of strong selection for a single offspring size, mothers produce a range of offspring sizes above a minimum value as a bet hedging strategy (Capinera 1979, Einum and Fleming 2002). This may particularly apply to $B.$ $violaceus$ given that it has dispersive larvae and mothers may be unable to predict the likely habitat of the offspring (Einum and Fleming 2002).

Most studies examining offspring size effects in plants and fish have found that the advantages of increasing per capita investment are strongly increased when intraspecific competition is increased (Stanton 1985, Einum and Fleming 1999, Seiwa 2000). In some species, mothers adaptively adjust the size of their offspring according to local conditions, including the probability of intraspecific competition (Fox et al. 1997, Kerrigan 1997). It would be interesting to see if colonies that were surrounded by other colonies (indicating higher incidences of competition) increase the size of their offspring in $B.$ $violaceus$. Regardless, it appears that depending on the local conditions, offspring size can have strong and pervasive effects on the subsequent performance of a colonial marine invertebrate. It appears that mothers have the potential to influence the competitive interactions of their offspring in a way that was previously unanticipated. We look forward to determining how other key demographic processes, such as predation and physical disturbance, affect the relationship between offspring size and performance in marine organisms.

thank the director and staff of the Oregon Institute of Marine Biology for help and support during this research.

LITERATURE CITED

Bagenal, T. B. 1969. Relationship between egg size and fry survival in brown trout *Salmo trutta* L. Journal of Fish Biology **1**:349–353.

Bennett, C. E., and D. J. Marshall. 2005. The relative energetic costs of the larval period, larval swimming and metamorphosis for the ascidian *Diplosoma listerianum*. Marine and Freshwater Behavior and Physiology **38**:21–29.

Bernado, J. 1996. The particular maternal effect of propagule size, especially egg size: patterns models, quality of evidence and interpretations. American Zoologist **36**:216–236.

Bervan, K. A., and B. G. Chadra. 1988. The relationship among egg size, density and food level on larval development in the wood frog (*Rana sylvatica*). Oecologia **75**:67–72.

Bingham, B. L., K. Giles, and W. B. Jaeckle. 2004. Variability in broods of the seastar *Leptasterias aequalis*. Canadian Journal of Zoology **82**:457–463.

Bullard, S. G., R. B. Whitlatch, and R. W. Osman. 2004. Checking the landing zone: do invertebrate larvae avoid settling near superior spatial competitors? Marine Ecology Progress Series **280**:239–247.

Buss, L. W. 1979. Bryozoan overgrowth interactions—the interdependence of competition for space and food. Nature **281**:475–477.

Buss, L. W. 1980. Competitive intransitivity and size frequency distribution of interaction populations. Proceedings of the National Academy of Sciences (USA) **77**:5255–5259.

Buss, L. W. 1990. Competition within and between encrusting clonal invertebrates. Trends in Ecology and Evolution **5**:352–356.

Capinera, J. L. 1979. Qualitative variation in plants and insects: effect of propagule size on ecological plasticity. American Naturalist **114**:350–361.

Clarke, A. 1993. Egg size and egg composition in polar shrimps (Caridea: Decapoda). Journal of Experimental Marine Biology and Ecology **168**:189–203.

Connell, J. H. 1961. Effects of competition, predation by *Thais lapillus*, and other factors on natural populations of the barnacle *Balanus balanoides*. Ecological Monographs **31**:61–104.

Connolly, S. R., and J. Roughgarden. 1999. Theory of marine communities: competition, predation, and recruitment-dependent interaction strength. Ecological Monographs **69**:277–296.

Dalby, J. E. 1995. Consequences of aggregated living in the ascidian *Pyura stolonifera*: evidence for non-contact intraspecific competition. Marine and Freshwater Research **46**:1195–1199.

Dunstan, K. P., and C. R. Johnson. 2003. Competition coefficients in a marine epibenthic assemblage depend on spatial structure. Oikos **100**:79–88.

Einum, S. 2003. Atlantic salmon growth in strongly food-limited environments: effects of egg size and paternal phenotype? Environmental Biology of Fishes **67**:263–268.

Einum, S., and I. A. Fleming. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. Proceedings of the Royal Society of London Series B **266**:2095–2100.

Einum, S., and I. A. Fleming. 2002. Does within-population variation in fish egg size reflect maternal influences on optimal values. American Naturalist **160**:756–765.

Engel, S., and J. R. Pawlik. 2000. Allelopathic activities of sponge extracts. Marine Ecology Progress Series **207**:273–281.

Etter, R. J. 1989. Life history variation in the intertidal snail *Nucella lapillus* across a wave-exposure gradient. Ecology **70**:1857–1876.

Fox, C. W. 2000. Natural selection on seed-beetle egg size in nature and the laboratory: variation among environments. Ecology **81**:3029–3035.

Fox, C. W., and T. A. Mousseau. 1996. Larval host plant affects fitness consequences of egg size variation in the seed beetle *Stator limbatus*. Oecologia **107**:541–548.

Fox, C. W., M. S. Thakar, and T. A. Mosseau. 1997. Egg size plasticity in a seed beetle: an adaptive maternal effect. American Naturalist **149**:149–163.

Gimenez, L., and K. Anger. 2001. Relationships among salinity, egg size, embryonic development and larval biomass in the estuarine crab *Chasmagnathus granulata*, Dana 1851. Journal of Experimental Marine Biology and Ecology **260**:241–257.

Gosselin, L. A., and P. Y. Qian. 1997. Juvenile mortality in benthic marine invertebrates. Marine Ecology Progress Series **146**:265–282.

Harvell, C. D. 1992. Inducible defenses and allocation shifts in a marine bryozoan. Ecology **73**:1567–1576.

Harvell, C. D. 1994. The evolution of polymorphism in colonial invertebrates and social insects. Quarterly Review of Biology **69**:155–185.

Havenhand, J. N. 1993. Egg to juvenile period, generation time, and the evolution of larval type in marine invertebrates. Marine Ecology Progress Series **97**:247–260.

Hunt, H. L., and R. E. Sheibling. 1997. Role of post-settlement mortality in recruitment of benthic marine invertebrates. Marine Ecology Progress Series **155**:269–301.

Hurlbut, C. J. 1991. The effects of larval abundance, settlement and juvenile mortality in the depth distribution of a colonial ascidian. Journal of Experimental Marine Biology and Ecology **150**:183–202.

Kaplan, R. H. 1992. Greater maternal investment can decrease offspring survival in the frog *Bombina orientalis*. Ecology **73**:280–288.

Keough, M. J. 1983. Patterns of recruitment of sessile invertebrates in two subtidal habitats. Journal of Experimental Marine Biology and Ecology **66**:213–245.

Keough, M. J. 1984*a*. Dynamics of the epifauna of the bivalve *Pinna bicolor*: interactions among recruitment, predation, and competition. Ecology **65**:677–688.

Keough, M. J. 1984*b*. Kin-recognition and the spatial distribution of larvae of the bryozoan *Bugula neritina* (L.). Evolution **38**:142–147.

Keough, M. J. 1989. Variation in the growth rate and reproduction of the bryzoan *Bugula neritina*. Biological Bulletin **177**:277–286.

Kerrigan, B. A. 1997. Variability in larval development of the tropical reef fish *Pomacentrus amboinensis* (Pomacentridae): the parental legacy. Marine Biology **127**:395–402.

Lack, D. 1947. The significance of clutch size. Ibis **89**:302–352.

Levitan, D. R. 1993. The importance of sperm limitation to the evolution of egg size in marine invertebrates. American Naturalist **141**:517–536.

Levitan, D. R. 1996. Predicting optimal and unique egg size in free spawning marine invertebrates. American Naturalist **148**:174–188.

Levitan, D. R. 2000. Optimal egg size in marine invertebrates: theory and phylogenetic analysis of the critical relationship between egg size and development time in echinoids. American Naturalist **156**:175–192.

Marshall, D. J., T. F. Bolton, and M. J. Keough. 2003*a*. Offspring size affects the post-metamorphic performance of a colonial marine invertebrate. Ecology **84**:3131–3137.

Marshall, D. J., and M. J. Keough. 2003*a*. Effects of settler size and density on early post-settlement survival of *Ciona*

intestinalis in the field. Marine Ecology Progress Series **259**:139–144.

Marshall, D. J., and M. J. Keough. 2003*b*. Variation in the dispersal potential of non-feeding invertebrate larvae: the desperate larva hypothesis and larval size. Marine Ecology Progress Series **255**:145–153.

Marshall, D. J., and M. J. Keough. 2004. Variable effects of larval size on post-metamorphic performance in the field. Marine Ecology Progress Series **279**:73–80.

Marshall, D. J., and M. J. Keough. 2005. Offspring size effects in the marine environment: a field test for a colonial invertebrate. Austral Ecology **30**:275–280.

Marshall, D. J., J. A. Pechenik, and M. J. Keough. 2003*b*. Larval activity levels and delayed metamorphosis affect post-larval performance in the colonial, ascidian *Diplosoma listerianum*. Marine Ecology Progress Series **246**:153–162.

McEdward, L. R., and F. S. Chia. 1991. Size and energy content of eggs from echinoderms with pelagic lecithotrophic development. Journal of Experimental Marine Biology and Ecology **147**:95–102.

McEdward, R. L., and L. K. Coulter. 1987. Egg volume and energetic content are not correlated among sibling offspring of starfish: implications for life-history theory. Evolution **41**:914–917.

Moran, A. L., and R. B. Emlet. 2001. Offspring size and performance in variable environments: field studies on a marine snail. Ecology **82**:1597–1612.

Nandakumar, K., and M. Tanaka. 1997. Effect of colony size on the competitive outcome of encrusting colonial organisms. Ecological Research **12**:223–230.

Okamura, B. 1984. The effects of ambient flow velocity, colony size, and upstream colonies on the feeding success of Bryzoa. I. *Bugula stolonifera* Ryland, an aborescent species. Journal of Experimental Marine Biology and Ecology **83**:179–193.

Podolsky, R. D. and R. R. Strathmann. 1996. Evolution of egg size in free spawners: consequences of the fertilisation–fecundity trade-off. American Naturalist **148**:160–173.

Rivest, B. R. 1983. Development and the influence of nurse egg allotment on hatching size in *Searlesia dira* (Reeve, 1846) (Prosobranchia: Buccinidae). Journal of Experimental Marine Biology and Ecology **69**:217–241.

Russ, G. R. 1982. Overgrowth in a marine epifaunal community: competitive hierarchies and competitive networks. Oecologia **53**:12–19.

Sebens, K. P. 1982. Competition for space: growth rate, reproductive output and escape in size. American Naturalist **120**:189–197.

Sebens, K. P. 1987. The ecology of indeterminate growth in animals. Annual Review of Ecology and Systematics **18**:371–401.

Seiwa, K. 2000. Effects of seed size and emergence time on tree seedling establishment: importance of developmental constraints. Oecologia **123**:208–215.

Smith, C. C., and S. D. Fretwell. 1974. The optimal balance between size and number of offspring. American Naturalist **108**:499–506.

Stanton, M. L. 1984. Seed variation in wild radish: effect of seed size on components of seedling and adult fitness. Ecology **65**:1105–1112.

Stanton, M. L. 1985. Seed size and emergence time within a stand of wild radish (Raphanus-Raphanistrum L). The establishment of a fitness hierarchy. Oecologia **67**:524–531.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, Oxford, UK.

Strathmann, R. R., M. F. Strathmann, and R. H. Emson. 1984. Does limited brood capacity link adult size, brooding and simultaneous hermaphroditism? A test with the starfish *Asterina phylactica*. American Naturalist **123**:796–818.

Underwood, A. J., and M. J. Keough. 2001. Supply-side ecology: the nature and consequences of variations in recruitment of intertidal organisms. Pages 183–200 *in* M. D. Bertness, S. D. Gaines, and M. E. Hay, editors. Marine community ecology. Sinauer, Sunderland, Massachusetts, USA.

Vance, R. R. 1973*a*. On reproductive strategies in marine benthic invertebrates. American Naturalist **107**:339–352.

Vance, R. R. 1973*b*. More on reproductive strategies in marine benthic invertebrates. American Naturalist **107**:353–361.

Wendt, D. E. 1996. Effect of larval swimming duration on success of metamorphosis and size of the ancestrular lophophore in *Bugula neritina* (Bryozoa). Biological Bulletin **191**:224–233.

Williams, M. D. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. Biological Reviews **68**:38–59.

APPENDIX A

The definitions of competition treatments (*Ecological Archives* E087-010-A1).

APPENDIX B

The values for model parameters (*Ecological Archives* E087-010-A2).