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Sources of variation in larval quality for free-spawning marine invertebrates: Egg size and the local sperm environment

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Summary

There has been growing interest in the effects of variation in larval quality on the post-larval performance of adult marine invertebrates. Variation in egg/larval size is an obvious source of variation in larval quality but sources of variation have received little attention. For broadcast spawners, larval size may vary according to the local sperm environment but the generality of this result is unclear. Here, we show that, for a solitary ascidian, a polychaete and an echinoid, larval size is affected by the concentration of sperm present during fertilization. Larvae that are produced at high sperm concentrations are smaller than larvae that are produced from eggs exposed to low sperm concentrations. We also show that for three ascidians and an asteroid, egg size increases with maternal body size. These differences in larval size are likely to affect larval and subsequent adult performance in the field. Given that sperm concentrations in the field can fluctuate widely, it is likely that larval quality in free-spawning marine invertebrates will also vary widely.

Key words: Egg size, maternal effects, fertilisation kinetics

Introduction

Recently, there has been growing interest in the effects of variation in larval quality on the performance of adult marine invertebrates. For a wide range of taxa, the quality of larvae that settle can affect post-metamorphic survival, growth, or reproduction (Pechenik et al., 1998; Moran and Emlet, 2001; Phillips, 2002; Marshall et al., 2003). There are several sources of variation in larval quality, although variation in the length of the larval period is perhaps the best studied. For a range of organisms, experimentally delaying metamorphosis results in a reduction in adult performance (Pechenik et al., 1998; Maldonado and Young, 1999). For lecithotrophic species, it is assumed that these delays in metamorphosis reduce the amount of energy stores available for post-metamorphic growth (Pechenik et al., 1998). In support of this hypothesis, Marshall et al. (2003) found that increasing larval activity (and hence energy consumption) had a greater impact on post-metamorphic performance than delaying metamorphosis alone. Given the results of these studies, it is reasonable to expect that other sources of variation in larval energetic content will also affect adult performance.

Variation in egg (and hence larval size) is one of the most obvious potential sources of variation in larval

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energetic content. Co-variation between egg size and maternal body size is a commonly identified factor affecting egg size in non-marine organisms (reviewed in Bernado, 1996; Sakai and Harada, 2001) and has been noted for a number of marine invertebrates (e.g., George, 1994; Bridges and Heppell, 1996; Bertam and Strathmann, 1998; Marshall et al., 2000; Marshall et al., in press). Variation in egg size may translate directly into variation in larval size, but in broadcast spawning marine invertebrates, this link may be mediated by the local sperm environment at the time of fertilisation.

When eggs are broadcast spawned, larger eggs present a larger "target" and are more likely to be contacted by sperm than smaller eggs if sperm are scarce in the field (Levitan, 1996). It has now been shown that the average size of eggs fertilised within a single clutch is dependent on the sperm environment in which fertilisation takes place (Levtian, 1996; Levitan and Irvine, 2001; Marshall et al., 2002; Podolsky, 2002). Marshall et al. (2002) found that when sperm concentrations were high, larger eggs became polyspermic whilst smaller eggs were successfully fertilised and developed into larvae. Conversely, when sperm concentrations were low, predominantly larger eggs were fertilised and smaller eggs were rarely contacted by sperm (Marshall et al., 2002). This represents a previously unrecognized source of variation in larval size that has the potential to affect subsequent performance.

Marshall et al. (2002) examined only a single species, but a mathematical model of fertilisation dynamics predicts that size-dependent fertilisation of eggs should occur for all free-spawning organisms (Styan, 1998) and others have also demonstrated the size-dependent fertilisation of eggs (e.g., Levitan and Irvine, 2001). Given that sperm environments vary widely in the field (Levitan, 1995; Marshall, 2002), the size of eggs that are fertilised (and hence larval size) has the potential to vary widely for a range of species. However, the size-dependent fertilisation of eggs has been questioned recently with the suggestion that egg accessory structures (such as follicle cells) and sperm chemoattractants may increase effective egg size, rendering differences in egg size trivial (Jantzen et al., 2001; Podolsky, 2001; but see Farley and Levitan, 2001; Levitan and Irvine, 2001).

Here we examine whether the size of larvae depends on the fertilising sperm environment for three species from different phyla, each with very different egg attributes. It is important to note that variation in larval size in broadcast spawners results from the interaction between the sperm environment and variation in egg size. In species where egg size is relatively uniform, the local sperm environment will have little effect on subsequent larval size. Conversely, highly variable broods of eggs have the potential to produce larvae of very different sizes, depending on the local sperm concentration. Therefore, we also examined intraspecific variation in egg size for a number of broadcast spawning species and tested whether egg size varied with maternal size.

Materials and Methods

Study sites and species

We collected sea urchins, *Arbacia punctulata*, from St. Petersburg beach, Florida, USA, on August 14, 2000, from a depth of 5 m. Individuals of the solitary ascidian *Styela plicata* were collected from the University of South Florida, St. Petersburg campus dock, on July 10, 2000, from a depth of 2 m. We collected another solitary ascidian, *Ciona intestinalis*, on March 22, 2000, and the sea star *Uniophora granifera* on October 29, 1999, from Pt. Wilson Pier, Victoria, Australia, at a depth of 6 m. We collected a third solitary ascidian, *Pyura fissa*, at a depth of 4 m from Edithburgh Jetty, South Australia, on October 7, 2000. We collected the serpulid polychaete *Galeolaria caespitosa* from the intertidal zone at Flinders Pier, Victoria, Australia, on April 25, 2002. We collected each species when most of the individuals of population were reproductively mature (as reflected by their gonad indicies; Marshall unpubl. data) and collected individuals of a range of sizes. We were concerned that some individuals may not have been totally reproductively mature, and therefore we only used individuals that produced eggs that were >95% viable (measured as number of eggs showing cell division after exposure to optimal sperm concentrations). Only a small (<10%) proportion of individuals had eggs that were not at least 95% viable and these were discarded. Importantly, smaller individuals of all species were at a similar state of reproductive maturity to larger individuals.

Arbacia punctulata produce eggs (~60 μm diameter) that are surrounded by jelly coats. The larvae are planktotrophic and reach the prism stage about 11 h after fertilisation. *Ciona intestinalis*, *Styela plicata* and *Pyura fissa* produce eggs that are ~150 μm in diameter, which develop into non-feeding tadpole larvae about 20 h after fertilisation. *Galeolaria caespitosa* produces 80 μm eggs that develop into planktotrophic trochophores about 17 h after fertilisation. *Uniophora*

granifera produces large (~500 μm diam.) eggs that develop into non-feeding larvae about 24 h after fertilisation.

Collection of gametes

For all three ascidian species, we removed the tunic of the ascidian and measured the (wet) weight of the visceral mass. For *Styela plicata* and *Pyura fissa* we collected sperm and eggs as in Marshall et al. (2000). For *Ciona intestinalis*, we made a small slit in the oviduct or sperm duct. We then sucked the eggs or sperm out using a fine glass pipette. We did not use the same individual to collect both eggs and sperm, to minimize the chances of self-fertilisation. To collect the gametes of *Arbacia punctulata* we dissected out the gonad and tore it into several pieces, filtered off any debris using a 100-μm mesh and collected the eggs using a pipette. To collect the gametes of *G. caespitosa* we removed individuals from their tubes using forceps and placed them in 5 ml vials of filtered (0.45 μm) seawater. Reproductively mature individuals of this species release gametes immediately upon being removed from their tubes.

To collect the eggs of *U. granifera* we injected 1 ml of 100 μM 1-methyladenine into each individual. About 30 mins after injection, individuals would begin typical spawning behaviour and begin shedding gametes. The eggs of this species are strongly, positively buoyant and we collected the eggs from the surface of the water using a syringe. We estimated the size of adult *U. granifera* by measuring the length (to the nearest mm) of the longest arm from the centre of the disc.

Measurement of eggs

For *Styela plicata*, *Ciona intestinalis*, *Pyura fissa* and *Uniophora granifera* we measured the size of eggs from individuals of a range of sizes. After collection, eggs were fixed with a few drops of formalin in seawater. Later the eggs were videotaped under a dissecting microscope. The video footage was digitised and 100 haphazardly selected eggs from each individual were measured. For the ascidian species, we measured the diameter of the eggs, not including follicle cells or the chorion, as neither will contribute to the energetic reserves of the larvae.

Production of larvae

For *Ciona intestinalis*, *Arbacia punctulata* and *Galeolaria caespitosa* we were interested in the effect of sperm concentration at fertilisation on larval size. For all three species, we exposed eggs of one individual to a wide range of sperm concentrations from another individual (see Marshall et al., 2000, for method and example of fertilisation curve). Hereafter, we refer to the embryos of one set of parents as a batch. Our focus was the characteristics of larvae fertilised at relatively high sperm concentrations and relatively low sperm concentrations, respectively. In free-spawning marine invertebrates, fertilisation success increases with initial increases in sperm concentration but decreases as sperm becomes very abundant due to polyspermy (Styan, 1998; Franke et al., 2003; for examples of fertilisation curves, see Marshall et al., 2000; Styan and Butler, 2000; Franke et al., 2003). Therefore, we only used zygotes from the lowest sperm concentration that produced ≈10% fertilisation success and zygotes from the highest sperm concentration that produced ≈20% fertilisation success. It should be noted that the maximum fertilisation success values for the eggs of each individual among the different species were around 95%. This high level of fertilisation success was achieved typically using an intermediate sperm concentration. Table 1 gives the approximate sperm concentrations that produced the desired levels of fertilisation success for each species. Whilst the exact concentration varied among batches, all were within the same order of magnitude. Sperm concentrations were estimated using a modified haemocytometer, using the mean concentration from three replicate counts.

For each of the three species we exposed the unfertilised eggs to the relevant sperm concentration for approximately 30 min. A subset of eggs was exposed to filtered seawater to control for errant fertilisations, and as a positive control, another subset was exposed to an optimal sperm concentration to ensure the eggs were viable. In any batches where the control eggs showed >1% fertilisation success or positive control eggs showed <95% fertilisation success, the batch was discarded. The eggs were then

Table 1. Approximate sperm concentrations used to produce differently sized larvae of *Ciona intestinalis*, *Arbacia punctulata* and *Galeolaria caespitosa*

Species	High conc. (sperm μl^{-1})	Low conc. (sperm μl^{-1})
Ciona intestinalis	1.0×10^5	10
Arbacia punctulata	1.0×10^6	100
Galeolaria caespitosa	2.0×10^7	2.0×10^3

rinsed in filtered (0.45μm) seawater on a Nitex mesh and left to develop in 30 ml of filtered seawater in polyethylene vials at room temperature (~20°C). For each batch of larvae at each sperm concentration we had three replicate vials of developing embryos. From each replicate vial at least 20 haphazardly selected larvae were measured. The data from these vials were pooled because the batch was the unit of replication for testing effects of sperm environment (*C. intestinalis*: n = 7 batches, *A. punctulata*: n = 4, *G. caespitosa*: n = 4).

Measurement of larvae

For *A. punctulata* we measured the diameter of gastrulae, about 2 h prior to the formation of the prism stage (~13 h after fertilisation). We only measured actively swimming gastrulae.

In *G. caespitosa*, trochophores were fully formed approximately 15 h after fertilisation. We measured the maximum length of the trochophore and the width of the trochophore at the ciliated band about 17 h after fertilisation.

For *C. intestinalis*, we measured the overall length of the tadpole larvae and the maximum width of the head. Pilot studies showed that these dimensions changed slightly within individuals during the period immediately after hatching. However, once the papillae had formed (about 20 h after fertilisation), overall larval length and maximum head width remained constant over time (Marshall, pers. obs).

For most of the species in this study, we collected individuals as close to the peak of their spawning season as possible. Consequently, we had only a short temporal window in which to collect specimens before they spawned so we were unable to collect data on both egg size and the effects of sperm concentration on larval size for each species and therefore addressed only one question for each species (depending on which question was of greater interest for each species).

Data analysis

For all comparisons of larval size we compared larvae produced at high and low sperm concentrations, respectively, using paired *t*-tests. Data were the means of replicate vials within each batch, and batches were the replicates for the test. Because we predicted that larger larvae would be produced at the low sperm concentration, all size comparisons were one-tailed.

Results

Effects of fertilising sperm concentration on larval size

The eggs of *C. intestinalis* took 19–20 h to develop into actively swimming larvae. Larvae that developed from eggs that were exposed to a high sperm concentration had ~10% narrower heads (paired *t*-test: $t = 3.932$, $df = 6$, $P = 0.004$; Fig. 1) and were ~7% shorter in overall length than larvae that developed from eggs that were fertilised at a low sperm concentration (paired *t*-test: $t = 2.907$, $df = 6$, $P = 0.014$; Fig. 1).

A. punctulata gastrulae that developed from eggs exposed to a high sperm concentration had a smaller mean diameter than gastrulae that developed from eggs exposed to a low sperm concentration (paired *t*-test: $t = 6.641$, $df = 3$, $P = 0.004$; Fig. 2). This translates into approximately a 15% average difference in volume.

G. caespitosa trochophores that developed from eggs that were exposed to a high sperm concentration were shorter than trochophores that developed from eggs exposed to a low sperm concentration (paired *t*-test: $t = 2.582$, $df = 3$, $P = 0.041$; Fig. 3) and a similar, though non-significant trend in the difference the width of trochophores fertilised under the different sperm concentrations (paired *t*-test: $t = 2.033$, $df = 3$, $P = 0.068$; Fig. 3).

Variation in egg size

Egg size increased with body size for all four species that were examined here. Mean egg diameter increased with maternal weight in *Ciona intestinalis* (regression test of slope: $R^2 = 0.261$, $P = 0.021$, n = 20;

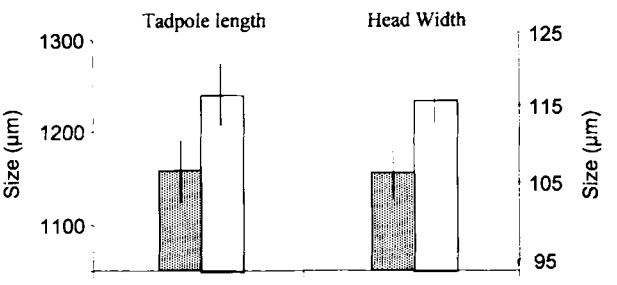


Fig. 1. Effects of different sperm environments during fertilisation on the size of *Ciona intestinalis* larvae. The mean size of larvae from eggs fertilised using a high sperm concentration is represented by shaded bars and mean size of larvae from eggs fertilised at a low sperm concentration is represented by open bars. Note that the error shown represents the standard deviation of the difference between treatments using a paired *t*-test.

Fig. 4a). Within broods, the mean coefficient of variation in egg diameter was 4.2% (± s.e. = 0.24).

Mean egg size increased with maternal weight in *Styela plicata* (regression test of slope: $R^2 = 0.340$, $P = 0.022$, n = 15; Fig. 4b). There was approximately a 20% difference in volume between the eggs produced by the largest and smallest individual. Within broods, the mean coefficient of variation in egg diameter was 3.9% (± s.e = 0.18).

Mean egg size increased with maternal weight in *Pyura fissa* (regression test of slope: $R^2 = 0.829$, $P = 0.012$, n = 6; Fig. 4c). This resulted in approximately a 15% difference in volume between the eggs produced by the largest and the smallest individuals. Within broods, the mean coefficient of variation in egg diameter was 3.4% (± s.e = 0.22).

Mean egg size increased with maternal size in *U. granifera* (regression test of slope: $R^2 = 0.62$, $P = 0.001$, n = 13; Fig. 4d). This relationship resulted in approximately a 56% difference in egg volume between the largest and smallest individuals that were examined. Egg size was variable within broods (mean coefficient of variation in egg diameter ± s.e. = 6.45% ± 0.39).

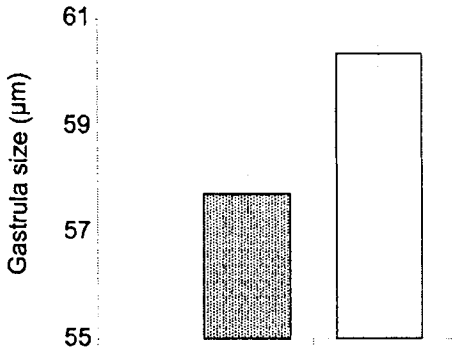


Fig. 2. Effects of different sperm environments during fertilisation on the size of *Arbacia punctulata* larvae. The mean size of larvae from eggs fertilised at a high sperm concentration is represented by a shaded bar and the mean size of larvae fertilised at a low sperm concentration is represented by an open bar. Note that the error shown represents the standard deviation of the difference between treatments using a paired *t*-test.

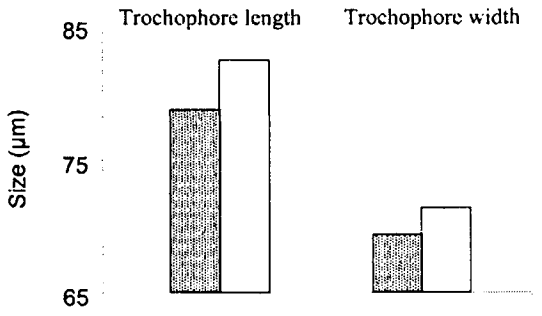
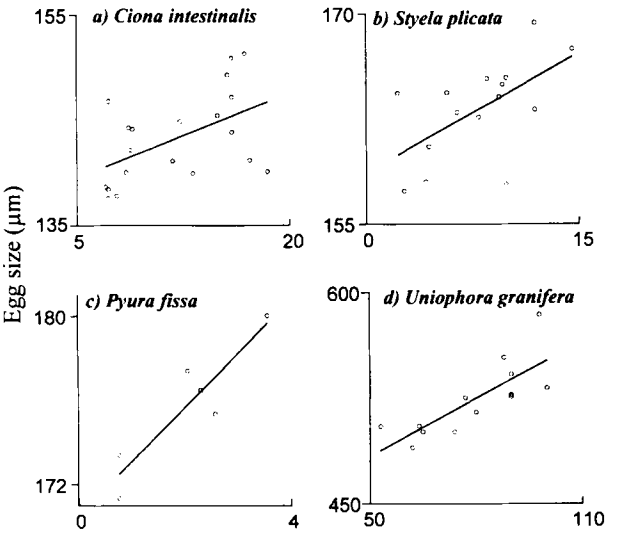


Fig. 3. Effects of different sperm environments during fertilisation on the size of *Galeolaria caespitosa* larvae. The mean size of larvae from eggs fertilised using a high sperm concentration is represented by shaded bars and mean size of larvae from eggs fertilised at a low sperm concentration is represented by open bars. Note that the error shown represents the standard deviation of the difference between treatments using a paired *t*-test.

Discussion

We found that larval size depended on the concentration of sperm in which the eggs were fertilised for all of species we tested. Larvae produced from eggs fertilised in a relatively low sperm concentration were larger than larvae produced from eggs fertilised in a high sperm concentration. This result was consistent



[1]For each of the ascidian species, maternal body size was measured as visceral mass (g), for *Uniophora granifera*, maternal body size was measured as arm length (mm)

Fig. 4. (a) Relationship between mean egg diameter and maternal body mass for *Ciona intestinalis* collected from Pt. Wilson, Victoria. (b) Relationship between mean egg diameter and maternal body mass for *Styela plicata* collected from St. Petersburg. (c) Relationship between mean egg diameter and maternal body mass for *Pyura fissa* collected from Edithburg Jetty, South Australia. (d) Relationship between mean egg diameter and maternal body size for *Uniophora granifera* collected from Pt. Wilson, Victoria. Each point shows the mean of 100 eggs from a given parent.

across the three phyla examined and regardless of the various egg attributes that could mitigate the effects of differing sperm concentrations. The eggs of *C. intestinalis* have large follicle cells surrounding the chorion and produce sperm chemoattractants (Bolton and Havenhand, 1996). It has been suggested that chemoattractants may make trivial the effects of egg size on fertilisation by substantially increasing the effective egg size (Jantzen et al., 2001). Echinoid eggs have jelly coats that may reduce the effect of egg size on the likelihood of fertilisation (Podolsky, 2001). However, Levitan and Irvine (2001) showed that whilst jelly coats may have an effect, the effect of egg size alone on fertilisation was far greater. Furthermore, Farley and Levitan (2001) demonstrated that whilst egg jelly coats may increase the rate of collisions between eggs and sperm, they may decrease the ratio between fertilisations and sperm–egg collisions. From our results, we cannot rule out the possibility that accessory structures or chemoattractants do affect the fertilisation kinetics of eggs. However, in the laboratory at least, the size of eggs that are fertilised (and hence the size of larvae that are produced) depends strongly on the sperm concentration that is present, despite these other mechanisms. It appears then, that the differential fertilisation of differently sized eggs under varying sperm conditions will occur in a range of species.

For the four species of marine invertebrate examined in this study, larger mothers produced larger eggs. The variation between mothers of different sizes was most extreme in the starfish *U. granifera* with a 50% difference in egg volume between the smallest and largest individuals, with similar ranges for the three ascidians. There are now a number of documented cases of maternal size: offspring size covariation including a colonial species of marine invertebrate (e.g., ascidians: Marshall et al., 2000; bryozoan: Marshall et al., in press; fish: Chambers and Leggett, 1996, but see Jones et al., 1996). The fact that egg size can vary with maternal size suggests that, in some species, the quality of larvae produced by individuals within a population will be influenced by the size structure of that population.

In each of the three species tested, there was approximately a 10–15% difference in the size (measured as volume) of larvae that were produced at the different sperm concentrations. It should be noted that the sperm concentrations used here were only an experimental convenience and greater differences in sperm concentration are likely to result in even greater differences in larval size (Styan, 1998). The differences in larval size observed here are likely to have a number of consequences for the performance of the larvae and subsequent juveniles, although these effects may vary between the planktotrophic and lecithotrophic species. If planktonic mortality rates are size-specific (Morgan, 1995), then larvae that come from larger eggs (i.e., those fertilised at low sperm concentrations) may have much higher survivorship than larvae from smaller eggs. For planktotrophic species (e.g., *G. caespitosa* and *A. punctulata* in our study), variation in original larval size (or per offspring maternal investment) may strongly affect the larval phase. For example, differences in larval size may lead to differences in maximum clearing rates (Hart, 1995) and egg size may affect size-at-age of feeding structures of planktotrophic larvae (Bertram and Strathmann, 1998; Hart, 1995). Larger larvae may also be able to change their form in response to food scarcity more quickly than smaller larvae or metamorphose sooner (George, 1999). Consequently it seems likely that the larvae that were produced at different sperm concentrations in this study would differ greatly in their survivorship and performance as larvae.

It is unclear how the differences in original larval size for the planktotrophic species observed in this study will carry through to the juvenile stage. In planktotrophs, maternal investment contributes a small proportion of total larval nutrition and juvenile energetic content is principally determined by larval feeding (Bertram and Strathmann, 1998). For lecithotrophic species (e.g., *C. intestinalis* and *U. granifera* in our study), maternal provisioning is the sole source of nutrition, and therefore, the variation in larval size we observed is likely to have profound consequences for juvenile performance. As mentioned above, differences in energetic content of settling larvae due to experimentally delaying metamorphosis result in reduced growth and reproduction in a range lecithotrophic species (Pechenik et al., 1998). Furthermore, initial studies show that larval size can have equally strong effects on survival, growth and reproduction of adult marine invertebrates (Moran and Emlet, 2001; Marshall et al., in press). In light of this, our study suggests that the sperm environment that is present when eggs are released can indirectly affect the survival or quality of settling individuals and could even carry through to affect adult performance. If the fertilising sperm concentration varies in the field, then the quality of larvae that are produced will also vary.

Measurements of sperm concentrations in the field are rare, but data do exist that suggest that sperm concentrations during natural spawning events can be extremely variable (Yund, 2000). Marshall (2002)

found that when the solitary ascidian *Pyura stolonifera* spawned naturally in the field, the fertilisation success of some individuals was sperm limited whilst in other individuals of the same population, fertilisation appeared to be limited by the presence of too much sperm. In other species of free-spawners, fertilisation success can vary substantially, indicating that among different broods of spawned eggs the sperm concentration can vary widely (Yund, 2000; Brawley, 1992). Our results suggest that the quality of larvae produced by these different broods will also vary accordingly.

Whilst sperm concentrations can vary widely in the field, populations that are at high densities are likely to create a higher concentration sperm environment than populations at lower densities (reviewed in Levitan, 1995). Therefore, at high population densities, larger eggs are more likely to become polyspermic than smaller eggs. Conversely, at low population densities, we predict that smaller eggs are less likely to be fertilised than larger eggs. We found that larger individuals produce larger eggs within three species of marine invertebrate. Others have found similar relationships between maternal body size and egg size (Marshall et al., 2000; Bridges and Heppell, 1996; Chambers and Leggett, 1996). If body size is negatively correlated with population density (e.g., Dalby, 1995), then it is possible that larger mothers (which presumably live in low-density environments) produce larger eggs in order to maximise their fertilisation success. A similar explanation has been invoked to account for differences in egg size among species of sea urchin that live in very different hydrodynamic (and hence sperm) environments (Levitan, 2002). Whilst highly speculative, it is possible that egg size is relatively plastic and can vary according to the likely sperm environment that spawning individuals will encounter. An interesting challenge will be to determine if the manipulation of population densities results in a change in the size of eggs that are produced.

Much discussion has focused on the evolution of egg size and its effects on offspring performance (Bernado, 1996; Moran and Emlet, 2001). For the species examined here, we have shown that variation in the sperm concentration at fertilisation results in variation in larval size. We would expect that this effect occurs in any free-spawning marine invertebrate that produces eggs that vary in size (Styan, 1998). Therefore, we suggest that the method for manipulating fertilised egg size described here may be useful in addressing some of the questions surrounding the effects of egg size. This method has the advantage of using natural variation in egg size and produces different sized offspring of identical parentage.

We are beginning to recognise that variation in larval quality results in variation in the post-settlement performance of marine invertebrates. Here, we have identified an additional, important source of variation in larval size for free-spawning marine invertebrates. Whilst the upper and lower limits of larval size will be determined by the size range of eggs produced by free-spawning mothers, the mean size of larvae that are produced within these bounds will be determined by the sperm environment.

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