

The relationship between egg size and fertilization success in broadcast-spawning marine invertebrates

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Synopsis Egg size is a critical life-history trait because it can profoundly influence offspring fitness and the number of offspring that can be produced. Recently, interest has grown in how egg size influences fertilization rate and in turn how sperm availability might influence the evolution of egg size among broadcast-spawning marine invertebrates. In this article I review the empirical evidence on the ways in which egg size and egg accessory structures influence fertilization and theoretical models of the ways sperm availability might influence the evolution of egg size. Evidence suggests that egg size does influence the collision frequency with sperm, and models suggest that sperm availability can influence selection on egg size. Sperm availability appears to be one of the several factors that influence optimal egg size in broadcast-spawning marine invertebrates.

Introduction

Fertilization is the first of many important stages in the life cycle of sexually reproducing organisms. The likelihood of fertilization is a function of two critical rates, the rate of sperm–egg collision and the rate at which those encounters result in a fusion event. Among broadcast-spawning marine invertebrates, the encounter rate of gametes is a function of the environmental conditions that control gamete dilution and dispersion, population traits of distribution, abundance and sex ratio of individuals, the adult traits of the reproductive effort, synchrony and spawning behavior, and the traits of the gametes themselves (reviewed by Levitan 1995, 1998a). These gamete traits include the velocity and behavior of sperm (Gray 1955; Vogel and others 1982; Levitan 1993, 2000a; Farley 2002; Kupriyanova and Havenhand 2002) and the size and receptivity of egg (Rothschild and Swann 1951; Vogel and others 1982; Cox and Sethian 1985; Levitan 1993, Palumbi 1999, Evans and Marshall 2005).

The influence of egg size (volume) on fertilization success is particularly interesting because egg provisioning is a critical life-history trait that influences postzygotic fitness (Vance 1973; Smith and Fretwell 1974; Strathmann and Vedder 1977; McEdward 1986; Sinervo and McEdward 1988; Jaekle 1995; Herrera and others 1996; Levitan 2000b). Not only does egg size influence the fitness of an individual

offspring, it also influences the number of offspring that can be produced. This trade-off between egg size and egg number can produce an optimal investment per egg that maximizes parental fitness (Smith and Fretwell 1974). This trade-off complicates our understanding of the role fertilization might play in shaping the evolution of egg provisioning (Levitan 1993, 1996a, 2000b; Podolsky and Strathmann 1996; Luttikhuizen and others 2004; Podolsky 2004). Examination of egg size–egg number trade-offs in broadcast-spawning organisms is particularly interesting because these organisms lack the postzygotic provisioning that can complicate the trade-off by adding other avenues of resource allocation (McEdward and Carson 1987). In this article I review the evidence and theory surrounding five questions about the relationship between egg size and fertilization among broadcast-spawning marine invertebrates:

- (1) Does egg size influence the rate of fertilization?
- (2) How do accessory structures influence fertilization success?
- (3) How do egg traits influence fertilization under natural conditions?
- (4) Do theoretical models predict that sperm availability can influence the evolution of egg size?
- (5) How likely is fertilization to play a major role in influencing variation in egg size?

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Does egg size influence the rate of fertilization?

Studies across species

Theoretical models of gamete kinetics suggest that the size of the egg should influence the probability of fertilization by increasing the target size for sperm (Rothchild and Swann 1951; Vogel and others 1982; Styan 1998). Support for this hypothesis was found in a comparison of three congeneric sea urchins that differ 5- to 6-fold in egg volume and require different concentrations of sperm to achieve 50% fertilization of eggs (Levitan 1993). The species with the smallest eggs, *Strongylocentrotus purpuratus*, requires the highest concentration of sperm, followed by *Strongylocentrotus franciscanus*, which has intermediate egg size and requires an intermediate concentration of sperm, and finally *Strongylocentrotus droebachiensis*, the species with the largest eggs and that requires the lowest concentration of sperm. Similar tests across species have been conducted with sea urchins in the genus *Echinometra*, with similar results; species with larger eggs require less sperm to achieve fertilization than do species with smaller eggs (Rahman and Uehara 2004). In contrast, evidence from two *Patiriella* species, a planktotrophic species with small eggs (*Patiriella regularis*) and a lecithotrophic species with large eggs (*Patiriella calcar*), found no difference in the amount of sperm required for fertilization (Styan and others 2005).

Correlations of egg size with fertilization across species have been criticized as rigorous tests because factors other than egg target size might differ with species and influence fertilization (Podolsky and Strathmann 1996). The receptivity of the egg surface to sperm collisions (Levitan 1993), the compatibility of a particular sperm with eggs (Palumbi 1999; Levitan 2002a; McCartney and Lessios 2002; Evans and Marshall 2005), and sperm swimming ability (Gray 1955; Levitan 1993) can all vary with species and confound the relationship between a particular gamete trait and fertilization. For instance among *Strongylocentrotus* congeners sperm velocity is inversely related to both egg size and sperm longevity (Levitan 1993). Sperm velocity can influence the probability of fertilization when collisions are likely (Levitan 2000a; Kupriyanova and Havenhand 2002), and presumably sperm longevity can influence the probability of fertilization if collisions are rare. This confounding of traits within a gamete (for example, sperm velocity and longevity) and across gametes (egg traits and sperm traits) highlights the risk of comparing even closely related species. For these reasons, comparative studies across species have not provided a conclusive test of the

egg-size hypothesis (Podolsky and Strathmann 1996). More comprehensive tests involving many species in a phylogenetic framework that consider the independent and synergistic influences of multiple gamete traits would be welcome.

Studies among females

There have been a number of tests examining performance of gametes from different individuals within a population. These studies attempt to avoid the confounding issues associated with investigating multiple species with their unique set of reproductive traits. Intraspecific tests of fertilization success as a function of mean egg size among females have been conducted in broadcast-spawning species from three distinct taxa (echinoids, Levitan 1996b; ascidians, Marshall and others 2000; gastropods, Huchette and others 2004). Two of these studies directly measured fertilization success as a function of mean egg size (Levitan 1996b; Huchette and others 2004), and the third found a relationship between egg size and adult size and a relationship between adult size and fertilization success (Marshall and others 2000). Both these direct and more indirect studies indicate a significant inverse relationship between mean female egg size and the amount of sperm needed to fertilize 50% of eggs. These studies demonstrate that females significantly differ in the sizes of the eggs they produce and that those size differences are correlated with the likelihood of fertilization.

Not every study has documented that females producing larger eggs garner higher fertilization rates. George and others (1991) examined how nutrition influences egg quality in the sea star *Luidia clathrata* by establishing two groups of individuals fed high and low rations. The eggs from these females were then collected and pooled into the two treatments groups and the low-food group had significantly larger eggs and showed a marked trend of reduced fertilization when exposed to pooled sperm from the same low-food treatment (21% versus 100%). Because the fertilization assay was not replicated, the eggs were exposed to only a single and unknown concentration of sperm, and eggs from the low-food treatment were only exposed to sperm from that same treatment (and vice versa); it is not easy to interpret these results. Several possibilities exist for this trend. First, the starved males might have produced less sperm. Second, the starved females might have produced low quality (but large) eggs. Third, and less likely, the large eggs from the low-food treatment might have experienced increased levels of polyspermy. Regardless of the explanation, this study does suggest

that a simple measure of egg size, independent of other factors (such as sperm concentration and egg quality), is not a good predictor of fertilization success.

Not all variation in fertilization success can be explained by egg size differences among females (for example, Levitan 1996b = 68% and Huchette and others 2004 = 64%, variance in fertilization success explained by egg size). Other factors such as accessory structures of eggs (see the section below) and intraspecific variation in gamete compatibility (Palumbi 1999; Evans and Marshall 2005) or other aspects of gamete quality may also contribute to variation in fertilization success.

Studies within females

One way to reduce the genetic differences among eggs that might contribute to compatibility differences or other genetic or maternal influences on egg size is to examine phenotypic variation in egg size within a single female. Egg size varies within a female, and this variation is correlated with fertilization success. Tests that compare the sizes of eggs released by a female before the addition of sperm and the sizes of the eggs that remain unfertilized after a limiting amount of sperm is added, such that only a fraction of eggs are fertilized, indicate that larger eggs are preferentially fertilized when sperm are limiting. In these studies, when higher concentrations of sperm are added, all eggs were fertilized. Therefore, small eggs are not unfertilizable, they are simply less likely to be fertilized under conditions of sperm limitation. These tests include echinoids (Levitan 1996b; Levitan and Irvine 2001; Podolsky 2001; Marshall and Keough 2003; Marshall and others 2004), ascidians (Marshall and others 2002; Marshall and Keough 2003), and polychaetes (Marshall and Keough 2003). In addition, Marshall and colleagues have noted cascading effects of conditions of sperm limitation: larger eggs are fertilized, leading to larger embryos and larvae, higher hatching success, and shorter development times than those of eggs fertilized under conditions with abundant sperm (Marshall and others 2002, 2004; Marshall and Keough 2003).

One potential confounding problem associated with using artificially spawned eggs from single females is that it is possible that smaller eggs are developmentally less mature compared with larger eggs. Although efforts are often taken to use mature individuals (for example, Huchette and others 2004) and gametes (for example, Franke and others 2002), and studies typically find that the vast majority of eggs are fertilizable given the optimal sperm concentration, there may be subtle developmental differences among eggs that are correlated with egg size.

The strongest case for the hypothesis that egg size influences fertilization success is that this effect is predicted by fertilization-kinetics models and this pattern is noted over a variety of organizational levels (among species, females, and eggs). Problems noted at one level (for example, confounding effects of comparing species, compatibility differences among females, or developmental variation within females) are less likely to be a problem at other levels. While many factors of gamete quality likely influence fertilization, the effect of egg size typically rises above these sources of unexplained variance to significance.

How do accessory structures influence fertilization success?

The target size that influences sperm–egg collisions may be influenced by accessory structures that could increase the effective size of the egg (Buckland-Nicks 1993; Havenhand, 1995; Levitan, 1995; Podolsky and Strathmann, 1996; Farley and Levitan 2001; Levitan and Irvine 2001; Podolsky 2001, 2002, 2004). These accessory structures include a number of extracellular structures found on a variety of taxa (reviewed by Podolsky 2004). In addition, chemicals released by eggs may attract sperm or stimulate their activity (Miller 1966, 1985; Jantzen and others 2001; Riffell and others 2004).

The most studied accessory structure is the jelly coat surrounding echinoid eggs. Jelly coats have been implicated in a number of processes critical to the fitness of the egg, including protection from shear forces, such as ejection of egg from the female (Thomas and Bolton 1999; Thomas and others 1999; Bolton and others 2000), increasing sperm activity levels (Ohtake 1976), preparing sperm for fertilization (Lillie 1914; Foltz 1995), conferring species specificity (Bohus Jensen 1953; Hagstrom 1956a; Riffell and others 2004), providing a barrier to polyspermy (Hagstrom 1956b), and increasing the effective target size of the egg (Farley and Levitan 2001; Levitan and Irvine 2001; Podolsky 2001, 2002, 2004).

Work on how jelly coats might influence fertilization began early in the past century. Experiments demonstrated that stripping echinoid eggs of their jelly coats lowered the percentage of eggs fertilized (Rothchild and Swann 1951; Hagstrom 1956a) but raised the rate of fertilization (Hagstrom 1956b; Hagstrom and Markman 1957). This result is consistent with the notion that jelly coats increase the rate of collisions but that, because it takes time for the sperm to penetrate this layer, the rate of fertilization is reduced.

Similar results have been noted in more recent studies. Farley and Levitan (2001) examined, directly

by microscopy, gamete kinetics in the sea urchin *Lytechinus variegatus* by quantifying sperm collisions with eggs with and without jelly coats and with plastic beads. As predicted, collisions were more frequently observed at higher sperm concentrations and with eggs with jelly coats. When collision frequencies were adjusted by surface area, collisions with plastic beads and those with eggs with and without jelly coats did not significantly differ. This result suggests that collision frequencies can be explained in this species by target size. Estimates of fertilization made over a short time interval (sperm were immobilized by the addition of KCl after 10 s) found that jelly coats increased the likelihood of fertilization, but not to the degree predicted by an increase in target size. This result might have arisen because sperm colliding with a jelly coat may be less likely to fertilize an egg compared with sperm colliding directly with the egg surface. Another possibility is that sperm were immobilized by KCl before they could penetrate the jelly layer.

Podolsky (2002) investigated fertilization of eggs with and without jelly coats in the irregular echinoid *Dendraster excentricus* by removing the jelly coats from subsamples of eggs by two methods: (1) an acid wash and (2) washing the eggs in a calcium-free buffer that weakened the jelly coat and then sieving the eggs through Nitex. Both methods produced decreases in fertilization success greater than those predicted by the decrease in target size. The difference between the actual and predicted effects of jelly-coat removal could be a result of egg damage during jelly-coat removal (Podolsky 2002) or because the jelly coat increases fertilization success by some other means in addition to increasing the physical size of the egg. Jelly coats might, for example, influence sperm behavior or facilitate fusion by preparing the sperm before contact with the egg-cell surface (Foltz 1995).

Podolsky (2001) examined the contribution of egg-cell size and jelly-coat thickness to fertilization success in *D. excentricus*, using the Lande and Arnold (1983) method of estimating the intensity of selection on correlated characters. The results were that the standardized selection gradient (intensity of selection as a function of standard-deviation units of size) under conditions of sperm limitation was similar for egg-cell size and jelly-coat thickness; larger sizes were selected in both cases. Podolsky's study also indicated that the effect of the size change was predicted by the differences in naturally varying target sizes of eggs released by a female. A similar study (Levitan and Irvine 2001) also indicated that eggs and jelly coats had similar standardized selection gradients but that the unstandardized selection gradient (actual units of volume)

was greater for egg cells than for jelly coats. Therefore, for a given volume of change, greater fertilization success is expected with an increase in egg-cell volume than with an increase in jelly volume but that, given the higher variability in jelly volume among eggs, the decreased effect of jelly coats is compensated for by the greater opportunity for selection. A related analysis indicated that the higher ratio of egg to jelly material would be selected for, suggesting that collisions with thick jelly coats may not be as effective as collisions with thinner jelly coats in terms of actual fertilization. Overall, the selection analyses by Podolsky (2001) and Levitan and Irvine (2001) measured selection on phenotypic variation. Estimates of how these traits might actually evolve require knowledge of the heritability of these traits. A comparison of the heritabilities of cell size and accessory structures of the egg would be worthwhile.

Egg and jelly-coat size can also vary across populations. *S. droebachiensis* demonstrates a strong pattern of increasing egg size with higher latitudes in Scandinavia (Hagstrom and Lonning 1967). A recent study has documented that females from different sites along this cline in Norway show characteristic differences in egg size and the thickness of the jelly coat surrounding the egg surface. Females at a northern site produce larger eggs and thinner jelly coats, while females at a southern site show the opposite trend. Fertilization success depended on total target size (egg plus jelly-coat diameter), egg size, and egg-to-target ratio. An increase in both total target size and egg size had a positive effect on fertilization success, while an increase in jelly-coat thickness exerted a negative effect, but the strength of these effects varied with the sperm concentration. Under conditions of extreme sperm limitation, the positive effect of total target size reduced the detrimental effect of a proportionally thicker accessory coat. At intermediate and high sperm concentrations, the positive effect of total target size was reduced and larger eggs with a thinner jelly had greater fertilization success (J. Marks, C. Biermann, and E. Heegaard, unpublished data).

It seems clear that jelly coats increase collision frequency and generally this results in higher levels of fertilization. However, most of the accumulated evidence suggests that jelly coats decrease the proportion of collisions that result in fertilization (Farley and Levitan 2001; Levitan and Irvine 2001; J. Marks, C. Biermann, and E. Heegaard, unpublished data).

A related mechanism for increasing target size is the release of chemical sperm attractants by eggs (Miller and King 1983; Jantzen and others 2001). In numerous taxa, but not all (see for example, Miller 1985; Farley

and Levitan 2001; Kupriyanova and Havenhand 2002), chemicals contained in eggs have been found to elicit changes in sperm swimming behavior or to increase the activity level of sperm (for example, Miller 1975, 1985; Jantzen and others 2001). One study demonstrated that either saturating the water with a chemotactic chemical, so that no chemical gradient toward the egg remained, or destroying the chemical altogether reduced sperm attachment rates and fertilization in abalone (Riffell and others 2004). No empirical studies have examined how these chemical gradients perform under natural levels of water flow. Models have suggested that sperm chemotaxis could produce a large increase in the effective target size of the egg (Dusenbery 2000; Jantzen and others 2001). Dusenbery's model suggested that this would result in selection for larger eggs, because larger eggs would establish a larger radius of chemical attraction. The relationship between egg size and the effective radius of chemical attraction is yet to be tested. Jantzen and others's model predicted selection for smaller eggs because a chemical target would be an inexpensive alternative to additional egg cytoplasm. This point is similar to the argument that relatively inexpensive jelly coats (Bolton and others 2000) would be selected for over additional egg cytoplasm (Podolsky and Strathmann 1996; Podolsky 2001, 2002). This issue is addressed below in the section on theoretical aspects of fertilization.

Overall, accessory structures appear to increase the target size of the egg and often increase the likelihood of fertilization, but no evidence indicates that these structures obscure the effects of egg-cell size on fertilization. The correlation of increased fertilization success with larger egg-cell size has been noted in taxa with egg accessory structures and sperm chemotaxis. These results, as supported by the analysis of correlated characters (Podolsky 2001; Levitan and Irvine 2001) and intraspecific/interpopulation studies of variation in egg and jelly effects (J. Marks, C. Biermann, and E. Heegaard, unpublished data), indicate that sperm are more likely to fertilize larger than smaller egg cells in the laboratory. The mechanistic explanation for this relationship is collision frequencies, which has been supported by direct observations (Farley and Levitan 2001). However, it is possible that there are other factors tightly correlated with egg size and related to egg quality that modulate this relationship.

Do differences in egg traits influence fertilization in the field?

Results from controlled laboratory experiments might or might not reflect the ways various gamete traits

influence fertilization success in the field. Differences in the volume of water and water movement, adult abundance, and behavior might increase or decrease the importance of any of these traits to fertilization. Selection can act on gamete traits only if variation in those traits influences fertilization under natural spawning conditions.

A series of experiments on sea urchins indicates that variation in gamete traits does influence fertilization in the sea. In the first experiment, a male and a female of *S. franciscanus* were induced to spawn in the laboratory, and the laboratory performance of those gametes was quantified as the amount of sperm required to fertilize 50% of the female's eggs. Subsets of those eggs and sperm were then taken to the field, mixed with a dye, released into the sea, and then recaptured after the sperm were too dispersed to further influence fertilization. Field performance was quantified as the amount of time sperm could disperse, before egg release, and still fertilize 50% of the female's eggs. The result was replicated over a variety of days with different pairs of individuals and at sites that differed in wave expose. The results indicated that more than 50% of the variance in field fertilization performance could be explained by laboratory fertilization performance. The variance in natural water-flow conditions did not swamp the influence of gamete traits on fertilization (Levitan 1996b).

This experiment was expanded (Levitan 1998b) to include two common congeners of *S. franciscanus* (*S. purpuratus* and *S. droebachiensis*). As in laboratory experiments on gamete performance in these three species (Levitan 1993), species differences in the ease of fertilization in the field was highly correlated with the egg size differences among them. The rank order of success was tightly correlated with egg size and matched theoretical predictions of field performance based on egg size. An additional set of trials was conducted that tested the hybrid fertilization of the eggs of *S. droebachiensis* with the sperm of *S. purpuratus*. Field fertilization performance of the hybrid cross was significantly different from that of the sperm donor but not from that of the egg donor, suggesting that these differences in field performance were a function of egg rather than sperm traits (Levitan 1998b).

The last experiment in this series was intended to reflect natural conditions more closely. Male and female sea urchins from one of the three sea urchin congeners were induced to spawn in the field at natural densities (Levitan 2002b). After 30 min of spawning, eggs were collected above each spawning female, and the distribution and abundance of all male and female sea urchins were recorded. The experiment was replicated 20 times for each species. In Barkley Sound, on

the west coast of Vancouver Island, the rank order of population densities and nearest-neighbor distances are related to egg size and fertilization performance of these species. *S. purpuratus*, which has the smallest eggs (and fastest but shortest-lived sperm) lives at the highest densities and has the highest average fertilization success. *S. franciscanus* has intermediate gamete traits and intermediate densities and fertilization success. *S. droebachiensis* has the largest eggs (and slowest but longest-lived sperm) and the lowest densities and the lowest average fertilization success. However, in an analysis of covariance that adjusted the mean fertilization success (least squared means) on the basis of the average values of the covariates (population attributes and water-flow conditions), the rank order of response was reversed; the species with the highest levels of fertilization (*S. purpuratus*) had the worst fertilization performance, and the species with the lowest levels of fertilization (*S. droebachiensis*) had the best fertilization performance at the mean covariate values. This result has been confirmed through experimental manipulations of population density. When *S. purpuratus*, which is normally highly clumped, was induced to spawn at the lower densities characteristic of the other two species, its fertilization success was much lower than those of the other two species at those densities (Levitan 2002b). Similarly, when *S. franciscanus* was induced to spawn at high densities, polyspermy resulted (Levitan 2004), even though it has not been observed in field assays of *S. purpuratus*, which spawns at those or even higher densities (Levitan 2002b, unpublished data). These results are consistent with the notion that species-specific gamete traits are adapted to the typical levels of sperm availability for each species and that egg-size differences are at least correlated with these patterns.

Although the abundances of these sea urchins are different in different parts of their ranges, *S. purpuratus* is generally highly clumped in intertidal and shallow subtidal environments, whereas *S. franciscanus* is more randomly distributed in larger but less clumped populations (reviewed by Levitan 2002b). *S. droebachiensis* tends to have lower abundances on the outer west coast of North America but can be found at higher densities at other locations along its circumpolar distribution (see for example, Bernstein and others 1981). It will be interesting to note how these gamete traits and fertilization performances vary in different regions, but, as with any adaptive trait, trying to match trait values to present ecological conditions requires the assumption that the present conditions have persisted for enough time to influence the traits in question. Historic densities of many species (Dayton and others 1998; Jackson and others 2001), including these sea

urchins (Estes and Palmisano 1974; Foster 1990; Estes and Duggins 1995; Estes and others 1998), may have been quite different in times past, making this assumption questionable. The behavioral tendency of these species to clump or disperse may be a more reliable assay for historic estimates of local sperm environments than larger-scale estimates of abundances.

In sum the empirical results suggest, first, that egg size is correlated with fertilization success in the laboratory within females, across females within species, and often across species. Second, this result is not obscured in species that have been shown to have accessory structures or sperm chemotaxis that might influence fertilization success. Finally, field performance of gametes under a range of flow conditions is sensitive to variation in egg traits, within and among species, and this variation is correlated with egg size.

Do theoretical models predict that sperm availability can influence the evolution of egg size?

The empirical evidence suggests that larger eggs are fertilized at a higher rate under conditions of sperm limitation. To determine whether selection favors an increase in egg size, it is worth considering the full consequences of variation in egg size, including both benefits and costs of increasing egg size. The former include decreased development time or increased survivorship; the latter include the additional resources that must be allocated per offspring, which entail either an increase in the total allocation to reproduction or a decrease in the number of offspring. A variety of optimality models that consider various costs and benefits of increasing egg size and assume different functional relationships between egg size and fitness have been constructed, and all of them suggest that variation in sperm availability can influence the evolution of egg size.

Sorting out the predicted effects of sperm availability on egg size has been complicated by the different assumptions used by models to describe the relationship between egg size and development time. This assumption is critical because it largely determines how offspring fitness is related to offspring size. Earlier models of marine invertebrate optimal egg size have assumed a negative linear relationship between egg size and development time (for example, Vance 1973; Podolsky and Strathmann 1996), which results in predictions for extremes in egg size (but see McEdward 1997). More recently, models have instead assumed a proportional relationship (Levitan 1993, 1996a, 2000b; Luttkhuizen and others 2004;

Podolsky 2004), which results in predictions for a suite of intermediate egg sizes. This latter assumption has empirical support from at least echinoids (Levitan 2000b). Further tests on the relationship between offspring size and development time, or more generally offspring fitness, would be valuable.

The first attempt to estimate the influence of sperm availability on egg size (Levitan 1993) combined the optimal offspring size approach advocated by Smith and Fretwell (1974) with a fertilization-kinetics model developed by Vogel and others (1982). Smith and Fretwell (1974) produced a graphical model to illustrate how the relationship between offspring size and offspring fitness could be used to predict the offspring size that maximized parental fitness. The premise of the model was that, if the relationship between offspring size and fitness were curvilinear, such that increasing offspring size brought diminishing returns, the tangent of the curve through the origin would predict the offspring size that maximized parental fitness. This approach, used with the Vogel and others (1982) gamete-kinetics model, which included egg target size, showed that increasing the level of sperm limitation produced progressively larger optimal egg sizes (Levitan 1993). From the available data on gamete-kinetic parameters noted in the three species of *Strongylocentrotus* sea urchins, the model predicted that the different estimated levels of sperm availability for each species could explain the 5-fold differences in egg sizes among these species (Fig. 1). The levels of and functional relationship between zygote size and mortality in this example were arbitrary, and the point was that sperm availability could influence the evolution of egg size rather than that any of these species was at the predicted optimal size.

More recently, the Smith–Fretwell approach was used to estimate, more generally, how sperm availability might influence the evolution of egg size (Luttikhuisen and others 2004). The results indicated (1) that incorporating egg target size and sperm availability increased the range of conditions under which intermediate egg sizes would be selected, (2) that variation in sperm availability influenced optimal egg size, and (3) that this result was insensitive to the various fertilization-kinetics models that assume different patterns of behavior by sperm that encounter eggs (for example, whether excess sperm stick to eggs or bounce off and are still able to fertilize eggs).

A different approach was advocated by Podolsky and Strathmann (1996) to estimate the influence of sperm availability on optimal egg size. They based their model on the framework developed by Vance (1973) to explain the different developmental modes noted in marine invertebrates. Vance's model predicted that

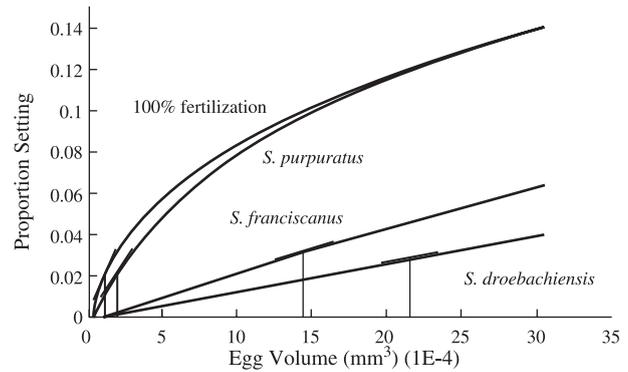


Fig. 1 Proportion of eggs that result in metamorphic individuals as a function of egg volume. The upper curve portrays an arbitrary size-dependent relationship, without considering fertilization success. The lower three curves adjust this curve by considering fertilization using species values for fertilization kinetics and predicted levels of sperm availability for three *Strongylocentrotus* sea urchin species. The tangent through each curve through the origin represents the optimal egg size that produces the maximum number of settling individuals for a given investment in egg material. The predicted optimal sizes match the empirical egg sizes for these species with these parameter values. Figure and details in Levitan (1993).

selection should favor an extreme in egg size: either some developmentally constrained minimum size or maximum size defined as an egg size with sufficient energy to nourish the egg through metamorphosis. The critical assumption of Vance's model that results in selection for extreme rather than intermediate egg size is that planktonic development time is a negative linear function of egg size (Fig. 2A and B). Podolsky and Strathmann (1996) modified this model to include the same gamete kinetics (Vogel and others 1982) as Levitan's (1993) model.

Vance's model results in binary predictions of egg size, shifting from small to large eggs as the level of planktonic mortality increases. Incorporating variation in fertilization success does not overcome the selective valley imposed by Vance's model, and Podolsky and Strathmann's model does not predict selection for intermediate egg sizes. However, Podolsky and Strathmann's model does predict that variation in sperm availability will change the level of planktonic mortality that triggers the switch from selection for small to selection for large egg sizes (Fig. 3). Therefore, although this study has been cited as evidence that sperm limitation might not influence selection on egg size (for example, by Havenhand 1995; Yund 2000; Randerson and Hurst 2001), the result of this model is that sperm availability does influence whether selection should favor small or large eggs. The general outcome, that only extremes

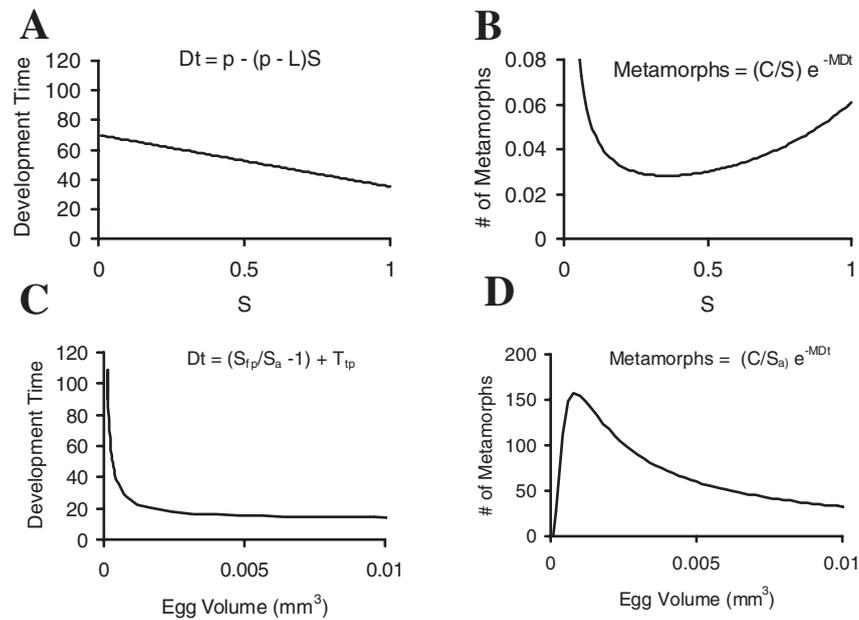


Fig. 2 The influence of the relationship between egg size and development time on the relationship between egg size and settlement success (the number of individuals that survive through metamorphosis given a constant amount of egg material). The top panels represent the negative linear relationship between egg size and development time (**A**) assumed by Vance (1973) and Podolsky and Strathmann (1996), which generates selection for extremes in egg size (**B**). The bottom panels represent the proportional relationship between egg size and development time (**C**) assumed by more recent work (Levitan 2000b; Farley and Levitan 2001; Podolsky 2004), which generates selection for intermediate egg sizes (**D**). In the top panel, development time (Dt) is a function of the time for development if nutrition is derived entirely from the egg ($L = 35$) or entirely from feeding ($p = 70$) and the egg size (0–1, with 1 being large enough to fully support development without feeding). In the bottom panel, the development time is a function of the actual volume of the egg (S_a) in relation to the empirical size of an egg that produces larvae that do not need to feed ($S_{fp} = 0.010306$, based on the facultative planktotroph *C. rosaceus*) and the time for metamorphosis of *C. rosaceus* ($T_{tp} = 13$ days). In both models, the number of metamorphs is a function of the total allocation to egg material (C), egg size, development time, and the daily mortality rate ($M = 0.15$). For details see Levitan (2000b).

in egg size are favored, is imposed by the confines of Vance's framework rather than by the effect of sperm availability on egg-size evolution per se.

Another first-principle model intended to explain variation in egg size among marine invertebrates assumed that development time was inversely but proportionately related to egg size (Levitan 2000b). This model matched (and Vance's linear assumption did not) the empirical data on extant echinoid species and also fit the evolutionary contrasts of egg-size and development-time changes estimated from a phylogenetic reconstruction of those species. Use of this proportional relationship eliminates the deep selective valley imposed by Vance's model and predicts intermediate optimal egg sizes (Fig. 2C and D). Variation in sperm availability and other factors such as variation in planktonic mortality, food availability, and temperature can therefore all influence optimal egg size in a continuous fashion. In a sensitivity analysis, egg size was most sensitive to changes in temperature; the other factors (mortality, food, and fertilization) were more similar in their sensitivity,

but optimal egg size was least sensitive to variation in fertilization. The importance of these factors to the evolution of egg size depends on the sensitivity of each factor and how that factor varies within and across populations.

Farley and Levitan (2001) made slight modifications to this model to include the target-size effect and the lower energetic cost of jelly coats than of egg cytoplasm. This modeling effort indicated that the presence of the jelly coat shifted the optimal egg size to a smaller size but that the sensitivity of optimal egg-cell size to changes in sperm availability remained the same. This study also included the possibility of developmental failure at high sperm concentrations, caused by polyspermy. Polyspermy has been demonstrated at high sperm concentrations in the laboratory (Schuel 1984; Styan and Butler 2000) and in field manipulations of spawning adults at high densities (Franke and others 2002; Levitan 2004). The model predicted that, if the fertilization kinetics allowed for polyspermic interactions (Styan 1998), optimal egg size would show an increased sensitivity to sperm availability, because

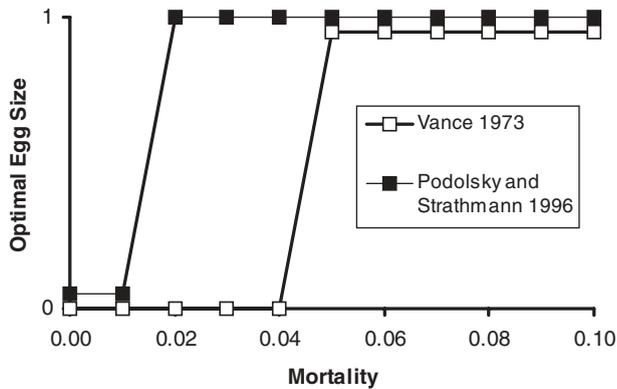


Fig. 3 Optimal egg size with (Podolsky and Strathmann 1996) and without (Vance 1973) considering the relationship between egg size and fertilization success as a function of planktonic mortality using the modeling framework developed by Vance (1973). When considering fertilization, the predicted evolutionary transition to larger eggs occurs at a lower level of mortality. For details see Podolsky and Strathmann (1996).

instead of pushing optimal size to an asymptote at high sperm concentrations, selection would favor smaller eggs (and fewer sperm–egg collisions) under conditions promoting polyspermy (Farley and Levitan 2001).

Podolsky (2004) also used the proportional relationship between development time and egg size (Levitan 2000b) to model how various permutations of gamete kinetics and egg traits might influence selection on egg size. He noted that in every case intermediate optimal egg sizes were predicted and that those sizes shifted with sperm availability. The degree of these shifts depended on the assumptions of the model.

In general, Podolsky noted an increased sensitivity of optimal egg size to variation in sperm availability when polyspermy and jelly-coat inefficiency (the tendency to increase collisions but to impede subsequent fertilization) were considered and a decreased sensitivity when jelly coats, variance in fertilization, and egg sinking rates were considered.

In contrast to Levitan's (2000b) predictions, Podolsky (2004) suggested that by considering jelly coats there would be a decrease in the sensitivity of optimal egg size to changes in sperm availability. Podolsky reasoned that the difference between the two model predictions was that he considered relatively long sperm–egg contact time intervals (10 min) while Levitan considered a shorter time intervals (10 s) and that the sensitivity of optimal egg size to sperm was increasingly dependent on jelly-coat thickness as contact time increased.

A reexamination of this model indicates that the change in sensitivity of egg size to sperm availability is a function of changing the parameter space examined, rather than simply considering jelly coats (Fig. 4). Podolsky is correct that, when all parameter values remain constant except for sperm–egg contact time, increasing the sperm–egg contact time decreases the sensitivity of egg size to sperm concentration when jelly coats are considered (Fig. 4A and B). However this sensitivity is restored, at these long contact time intervals, when egg concentration is adjusted such that the sperm–egg ratio is varied over a similar range across these different sperm–egg contact time intervals (Fig. 4B and C). The reason for these changes in sensitivity is that when sperm–egg contact time is shifted to longer intervals, it also shifts the range of sperm concentrations at which fertilization varies (when sperm have a longer time to find eggs, they can do so at lower sperm concentrations). This shift in the range of relevant sperm concentrations changes the ratio of sperm to eggs at those sperm concentrations. When the sperm–egg ratios are restored, the sensitivity is also restored. One can argue about the relevance of particular parameter values; however, it is evident that shifts in sperm–egg contact time to longer intervals do not necessarily decrease the sensitivity of optimal egg size to sperm availability. Under the parameter values examined here optimal egg–cell volume varied more than 11-fold at the lowered sensitivity (long sperm–egg contact time and high egg concentration) and varied nearly 17-fold at the higher sensitivity (short sperm–egg contact time and high egg concentration or long sperm–egg contact time and low egg concentration). In any of these scenarios, optimal egg diameter varied over the majority of empirically noted egg sizes among planktotrophic echinoids (between 80 μm and either 180 or 200 μm , depending on the parameter values chosen).

Egg sinking was modeled such that larger eggs sank faster than smaller eggs according to Stokes's equation (Vogel 1981), a pattern, Podolsky (2004) predicts, that would decrease the sensitivity of egg size to variation in sperm availability. The reasoning was that although larger eggs would sink faster and have a higher chance of encountering sperm as they passed through the sperm cloud, those eggs would also sink out of the sperm cloud faster and therefore have lower sperm–egg contact time. This model assumes that eggs must be lifted off the surface of the spawning animal and that this lifting force then ceases such that the eggs might fall back down out of the sperm plume before fertilization (or before the sperm plume dissipates to a sperm concentration at which fertilization is unlikely). This assumption might not be reasonable. During natural

spawning events, sperm are more concentrated near the bottom, where the spawning occurs (Hamel and Mercier 1996). If larger eggs sink faster than smaller eggs, then they accrue the double benefit of increased relative movement of eggs (which would increase sperm collisions) and movement into higher concentrations of sperm. Under these assumptions, shifts to larger egg sizes would have increased rather than decreased benefits for fertilization.

The last factor Podolsky analyzed that reduced the sensitivity of egg-size evolution to sperm availability was variation in sperm availability. While variation in sperm availability may influence selection on egg size, it is hard to estimate the relative importance of this variance, without a comparable examination of how variance in the other suggested factors (for example,

planktonic and post-settlement survivorship, water temperature, food availability) also temper selection.

These different optimality models all suggest that to some degree sperm availability influences optimal egg size. Although models have not been constructed that consider the effect and energetic content of chemical attractants, there is no reason to expect the predictions to qualitatively change; accessory structures and chemical attractants may alter the parameter space where sperm are limiting, but these traits are not likely to eliminate selection on egg size. Continued tests of the assumptions and further refinement of these models will be helpful, but ultimately the optimality approach must be matched with estimates of genetic variation and variation in the factors (sperm availability, mortality, food availability, etc) that exert selection on these traits.

The importance of sperm availability to the evolution of egg size in marine invertebrates

The likelihood that sperm availability influences the evolution of egg size depends on a number of factors, including the sensitivity of egg size to variation in sperm availability and the heritability of gamete traits including egg size, egg accessory structures, and sperm traits that might influence fertilization success, and on whether diversifying selection favors different egg size

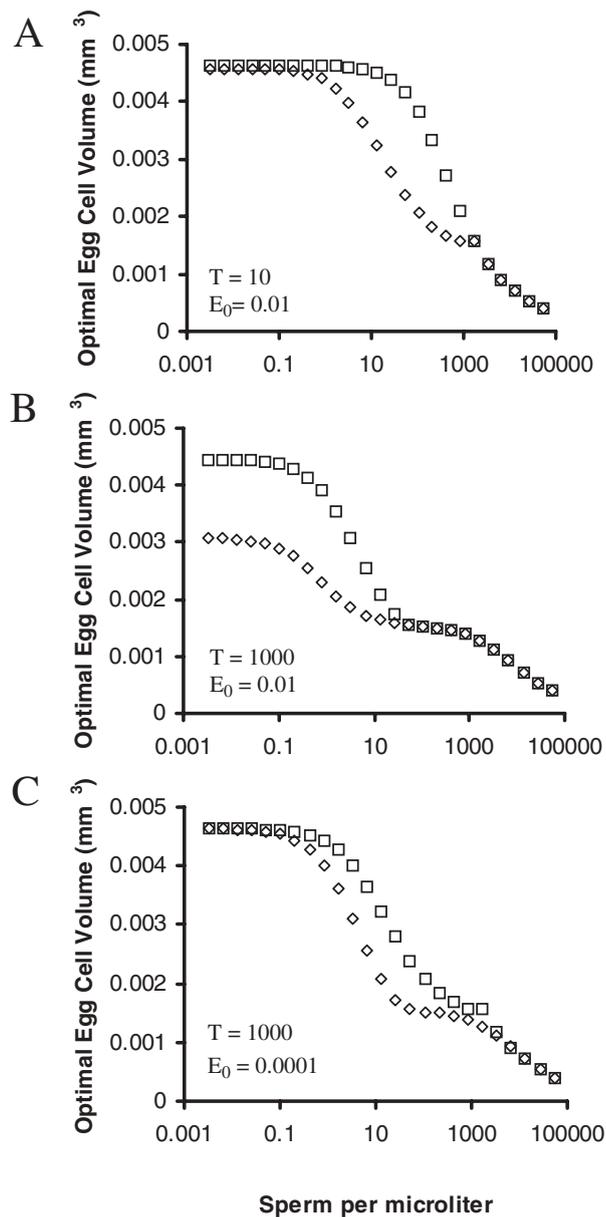


Fig. 4 Optimal egg-cell size as a function of sperm concentration using the approach of Levitan (2000a, 2000b) and Podolsky (2004). Model incorporates the fertilization-kinetics model developed by Styan (1998), the relationship between egg size and development time developed by Levitan (2000b), and the relationship between development time and mortality used by Vance (1973). Mortality rate (0.15 per day), time to polyspermy block (2 s), fertilization efficiency (0.05), and energetic cost of egg cell (207.5) and jelly (2.9) are all held constant. In all panels the square symbols represent optimal egg cell size without jelly coats and the diamond symbols represent the optimal egg-cell size, when jelly coats are also considered and optimized. The point where the two symbols converge at high sperm concentrations represents the threshold where polyspermy selects for a zero-sized jelly coat. The difference between panels A and B is that sperm-egg contact time is increased from 10 to 1000s at a constant egg concentration. The difference between panels B and C is that the egg concentration is reduced from 0.01 to 0.001 per μl to restore the egg-sperm ratios to the ratios present in panel A. The relevant sperm-egg ratios change by shifting the contact time because longer contact time intervals allow sperm to fertilize eggs at lower concentrations (note that the point of convergence occurs near 1000 sperm per μl in panels A and C and near 10 sperm per μl in panel B).

values in different populations or species. The optimality models reviewed here address only the first issue and suggest that sperm availability, along with a variety of other postzygotic and post-settlement of many factors, potentially influences egg-size evolution. The numerous selective forces on egg size are not mutually exclusive, and all of these factors contribute to the shape of the relationship between egg size and fitness that determines optimal egg size. Although one factor may have a larger influence on this shape than do others, the factors in which populations or species differ are those that will determine how egg size evolves. The evidence for biogeographic trends in egg size can provide some insight into how variation in selection may influence egg size.

One of the oldest recognized patterns of interspecific egg size among marine invertebrates is the Thorson's rule. Thorson (1950) noted that marine invertebrate taxa living at higher latitudes or at depth are more likely to have larger eggs and nonplanktonic larval forms than are those at more tropical latitudes or in shallower water. A variety of hypotheses have been invoked to explain this pattern, including gradients in planktonic predation, food availability, and water temperature (reviewed in Havenhand 1995; Levitan 2000b). Regardless of the mechanism, numerous counter examples have been noted, and wide interspecific variation in egg size and development mode are observed within a latitude and depth (Emlet and others 1987; Strathmann 1987; Pearse and others 1991). Similarly the Pacific members of geminate species pairs that live on opposite sides of the Isthmus of Panama tend to have smaller eggs than the other members found in the Caribbean region (Lessios 1990; Moran 2004). Again, variation in the planktonic environment has been invoked as the selective agent, and despite the trend interspecific variation in egg size is noted within each coast of Panama (Emlet and others 1987; Lessios 1990; Moran 2004). These patterns suggest that many factors concurrently influence selection on egg size and that, when one factor varies across regions, the result is a shift in egg sizes across regions, but not a convergence of egg size within each region.

Although variation in the planktonic environment might explain mean shifts in egg size across regions, it seems less likely to explain the wide variation in interspecific egg size noted within regions and spawning seasons. Invoking phylogenetic constraints as the reason for non-convergence of egg sizes within a region does not seem to be a universal explanation as, in at least echinoids, there is a wide variation in egg sizes between closely related species and phylogenetic corrections does not alter the relationship between egg size and development time (Levitan 2000b).

These findings suggest that egg size can be a labile character, unconstrained by phylogeny.

Variation in prezygotic and post-settlement factors seem more likely to explain interspecific variation in egg size within a region. Although many cooccurring marine invertebrate embryos and larvae may share the same planktonic environment during a spawning season (Harrison and others 1984; Strathmann 1987), even closely related taxa probably differ in prezygotic and post-settlement environments. The spawning environment can be different in intertidal and subtidal environments, in clumped and dispersed species, and in common and rare species. These gradients should all influence the level of sperm availability. Similarly, variation in juvenile and adult growth and survivorship will alter selection on optimal offspring size, as suggested by the trend toward larger eggs and by brooding strategies in species with smaller body size among closely related species (Strathmann and Strathmann 1982). Theoretical models have suggested that adult nutritional conditions can also influence optimal egg size (Sakai and Harada 2005).

Some selective agents are likely to be less important within particular mating strategies. For example, prezygotic factors are less likely to be important among species with internal fertilization (Podolsky and Strathmann 1996). Similarly postzygotic factors are less likely to be an important factor in taxonomic groups with similar sizes at metamorphosis (Levitan 2000b). No one factor is likely to explain patterns of egg size across all taxa and reproductive modes.

Studies that consider local adaptation of egg size within a broadcast-spawning species are rare. This is likely because broadcast spawners tend to disperse for relatively long periods during development, and this generally disrupts genetic population structure compared with reproductive strategies where fertilization is internal and dispersal is more limited (Palumbi 1995). However, a nice example is found in a study of the bivalve *Macoma balthica* that demonstrates genetic subdivision along the coast of Northern Europe (Luttikhuisen 2003). This variation is associated with differences in egg size among populations and these egg size differences are highly correlated with adult density and likely sperm availability. Differences in egg size do not seem to be a result of latitude or nutritional differences among populations and provide an excellent opportunity to test hypotheses concerning egg-size evolution and, specifically, hypotheses that consider sperm availability (Luttikhuisen 2003).

In sum, the available evidence and theory suggests that among broadcast-spawning taxa, sperm availability has the potential to influence selection on egg size. Given the highly variable nature of sperm availability

across rare and common species, and the various adaptations noted among species to increase encounter rates (and decrease polyspermy), such as adult aggregative behavior, synchronous spawning, and traits of the gametes themselves (Levitan 1995, 1998a, 1998b), it seems likely that sperm availability is one of many factors that has influenced the evolution of egg size.

There are many avenues for future work. These include broad taxonomic comparisons of gamete traits, fertilization rates and perhaps estimated sperm availability within a phylogenetic context to provide a more robust pattern of how gamete traits evolve. These would include comparisons both within gametes (for example egg size and receptivity or sperm velocity and longevity) and across gametes (do egg and sperm traits co-evolve differently under sperm limited versus sperm competitive conditions). Second, multigenerational selection experiments could examine how gamete traits and fertilizability evolve and the constraints on this evolution, under different levels of sperm availability. This would require developing marine invertebrate model systems with rapid generation times. Finally, there could be a detailed examination of the relative influence of gamete phenotype and egg–sperm recognition on fertilization rates within and among species. This may provide insight into variation in fertilization rates both within and among species and how this variation may influence the formation of reproductive isolation.

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