



Effects of egg size reductions on development time and juvenile size in three species of echinoid echinoderms: Implications for life history theory

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ABSTRACT

Life-history models for marine invertebrate animals assume a strong correlation between the size of the egg and the time from fertilization until metamorphosis. This assumption is supported by comparative data across a wide range of phyla. However, the exact form of the relationship between egg size and development time has a strong effect on the predicted outcomes of life-history models and has been the subject of much debate. Comparative data suggest that as egg size increases the effect of egg size reductions on development time will decrease and the effects of size reductions on juvenile size will increase. I used blastomere separations to test the effects of a 50% reduction in egg volume on development time and juvenile size in three species of echinoid echinoderms (*Arbacia punctulata*, *Strongylocentrotus purpuratus*, and *Dendraster excentricus*) that develop from eggs of a range of sizes (80–124 μm). I also manipulated the food level given to developing embryos and larvae to investigate the potential interaction between the effects of egg size reductions and food availability. Larvae from halved zygotes took significantly longer to develop to metamorphosis than their whole size counterparts in all three species. In only one species was I able to detect a significant reduction in juvenile size for offspring developing from halved zygotes. When compared with similar manipulations in species with larger eggs (140–387 μm), egg size reductions have a stronger effect on development time in species with small eggs. As predicted, development time does not change linearly with egg size but instead increases exponentially as egg size is reduced. The relationship between egg size and juvenile size remains unclear. Further investigations into the factors, including egg size, that influence juvenile performance are warranted.

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1. Introduction

In most living organisms, including plants, insects, marine invertebrates, fish, amphibians, and lizards, the number of offspring produced in a reproductive bout increases as the level of parental investment per offspring decreases (Roff, 1992, 2002). In organisms that lack parental care, the size of the egg is often used as a proxy for investment per offspring and the trade-off is therefore between the size and number of eggs produced. This trade-off between offspring size and number is one of the most well studied aspects of life-history evolution (Smith and Fretwell, 1974; Stearns, 1992).

Free-spawning marine invertebrates are a diverse group of animals that frequently lack parental care. For these animals, as egg size decreases, fecundity increases but so does the length of time required to complete development. An increase in the length of time spent developing is thought to be disadvantageous due to the high rates of mortality experienced by the developmental stages of marine invertebrates (see reviews by Morgan, 1995; Rumrill, 1990;

Vaughn and Allen, 2010; Young and Chia, 1987). The fecundity advantage of small egg size may be entirely offset by losses due to mortality even under modest rates of larval mortality (Strathmann, 1985). This trade-off between fecundity and time (known commonly as the fecundity-time hypothesis) is a central component of models of life-history evolution in marine invertebrates (Caswell, 1981; Christiansen and Fenchel, 1979; Levitan, 2000; McEdward, 1997; Vance, 1973a,b). The original predictions of this class of models suggested that only the extremes of egg-size distributions would be evolutionarily stable (Christiansen and Fenchel, 1979; Vance, 1973a). However, more recent modeling efforts have predicted that an intermediate set of egg sizes would be favored, a result that is more in line with the distribution of egg sizes seen in nature (Levitan, 2000; McEdward, 1997).

The fecundity-time hypothesis and the models that are based upon it are most strongly supported by a large set of comparative developmental data for echinoid echinoderms (Emlet, 1995; Emlet et al., 1987). However, there are few experimental data to support the fecundity-time hypothesis. While egg-size manipulations have been used to study the life-historical consequences of changes in maternal investment for a range of animal species (reptiles: Sinervo, 1990; birds: Nager et al., 2000; insects: Fox, 1997; and echinoids:

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Alcorn and Allen, 2009; Hart, 1995; Sinervo and McEdward, 1988), echinoids are perhaps the group of animals where experimental reductions in egg size are most readily achieved due to the regulative nature of development and the well-known methods for manipulating embryos (Allen, 2008; Harvey, 1940; Horstadius, 1973; McEdward, 1988, 1996; Strathmann, 1987). In echinoids, the egg sizes of large numbers of sibling offspring can be manipulated simultaneously and these manipulated embryos are readily reared to metamorphosis and even sexual maturity under controlled laboratory conditions (Cameron et al., 1996).

Despite the relative ease with which echinoid embryos can be experimentally manipulated, few studies of the effects of egg-size reductions have successfully demonstrated that these reductions significantly increase development time. Three experiments manipulating egg size in echinoid echinoderms found that a 50% reduction in egg volume had little or no effect on development time in species with relatively large eggs (Allen et al., 2006; Emlet and Hoegh-Guldberg, 1997; Hart, 1995). More recently, it has been shown that in at least two echinoid species with smaller eggs, reductions in egg size significantly increase larval development time (Alcorn and Allen, 2009). Taken together, the results of these experiments suggest that for species with small eggs, increases in egg size reduce developmental periods while for species with large eggs, increases in egg size increase juvenile size (and presumably performance).

Interspecific comparisons in echinoids have shown that, for large-egg species, differences in egg size are not correlated with differences in development time (Emlet, 1995). One recent model based on this comparative data set suggests that an inversely proportional relationship between egg size and development time best explains the observed distribution of egg sizes (Leviton, 2000). In order to experimentally confirm the nature of the relationship between egg size and development time, I tested the relationship between egg size and development time in three species of echinoid echinoderms that develop from eggs ranging in size from 80 to 124 μm . The minimum egg size known for echinoids is 65 μm and the median egg size is 110 μm (Emlet et al., 1987). The range of egg sizes used in this study therefore closely approximates the lower half of the egg size distribution, unlike previous studies where egg diameter ranged from 140 to 400 μm . In order to test the relationship between egg size and development time, blastomere separations were performed at the two-cell stage for each of the three species, generating sibling whole and half size embryos. These embryos were then reared to metamorphosis and the effect of blastomere separation on development time was measured. Effects of this reduction in egg size on percent survival, juvenile size, and juvenile organic content were also measured.

2. Methods

2.1. *Arbacia punctulata*

Adult *A. punctulata* were collected in May 2003 from a shallow subtidal habitat in Beaufort, NC. They were immediately transported to Chapel Hill, NC where they were maintained in aquaria with recirculating artificial sea water (ASW; Instant Ocean, Aquarium Systems Inc., Mentor, Ohio) at 22 °C with a salinity of 35 ppt. Adults were induced to spawn by an intracoelomic injection of 1 ml of 0.5 M KCl. Concentrated sperm were collected by mouth pipet and kept on ice. Eggs were collected by inverting females over beakers of ASW and allowing the eggs to sink to the bottom. Prior to fertilization, eggs were rinsed with ASW to remove debris. Sperm were activated by dilution in ASW and then a small volume (~1 ml) of this suspension was added to the beaker of eggs. Eggs and sperm were gently stirred until >90% were fertilized. Fertilization percentage was estimated by examining 50 eggs under a compound

microscope. Eggs were scored as fertilized if the fertilization envelope (FE) had elevated around the egg.

Blastomeres were isolated at the two-cell stage following the method of Harvey (1940). Within 5 min of fertilization, eggs were shaken vigorously for 1 min and then allowed to settle. This vigorous shaking effectively removed the FE. Following removal of the FE, embryos were allowed to develop undisturbed in ASW for approximately one hour at 22 °C until they had completed first cleavage. Embryos were then placed in hypertonic ASW for 10 min in order to separate the two blastomeres. Individual blastomeres and unseparated embryos were then transferred to normal salinity ASW and placed in an environmental chamber at 27 °C where they were allowed to continue development. Approximately 16 h post-fertilization, embryos had reached the swimming blastula stage and were sorted by mouth pipet into two size classes (factor SIZE): whole (W) embryos that developed from unseparated embryos and half (H) embryos that developed from isolated blastomeres.

Embryos of each size class were counted out into batches of 250 and placed into 1 L culture containers for a total of 500 embryos L^{-1} . One of the size classes was stained with Nile Blue Sulfate to distinguish size treatments within each container. A small amount (< 0.0001 g) of Nile Blue Sulfate was dissolved in 50 ml ASW and then passed through a 0.02- μm filter to remove any undissolved particles. Embryos were then placed in the stain for approximately 1 hour. After staining, embryos were rinsed one time in ASW and then added to the culture container along with the unstained embryos. The size treatment that was stained was alternated in order to control for the effects of the stain (factor STAIN). Following sorting and staining, containers of embryos were placed back into the environmental chamber at 27 °C. Embryos were kept suspended using a stirring rack attached to a small motor that moved paddles at a rate of 10 strokes min^{-1} (Strathmann, 1987).

Every other day, from fertilization until metamorphosis, food was added to containers and the water was replaced. Cultures of the unicellular alga *Dunaliella tertiolecta* (UTEX Algal Supply, Austin TX) were maintained in autoclaved ASW with modified Guillard's f/2 medium (Florida Aqua Farms Inc.). Algae were separated from culture medium by brief centrifugation and were added to "high food" containers every other day at a concentration of 5 cells μl^{-1} . "Low food" containers were fed algae every other day at a concentration of 1 cell μl^{-1} (factor FOOD). This experimental set up was replicated for three male–female pairs (factor TRIAL) but for one trial not all combinations of SIZE, STAIN and FOOD were completed (Table 1).

After larvae began to develop visible rudiments, a small amount of gravel from aquaria housing adult *A. punctulata* was added to each culture container in order to induce metamorphosis in competent

Table 1

Experimental design for trials using *A. punctulata*, *S. purpuratus*, and *D. excentricus*. Trials represent cultures derived from separate male–female pairs. SIZE treatments are whole (W), and half (H). Underlined letters are stained treatments. Number of replicate jars is in parentheses.

<i>A. punctulata</i>					
TRIAL A		TRIAL B		TRIAL C	
HIGH FOOD	LOW FOOD	HIGH FOOD	LOW FOOD	HIGH FOOD	LOW FOOD
<u>WH</u> (2)	<u>WH</u> (2)	WH (2)	<u>WH</u> (2)	WH (2)	<u>WH</u> (2)
		<u>WH</u> (2)	<u>WH</u> (2)	<u>WH</u> (2)	<u>WH</u> (2)
<i>S. purpuratus</i>					
HIGH FOOD					LOW FOOD
<u>WH</u> (2)					<u>WH</u> (2)
<u>WH</u> (2)					<u>WH</u> (2)
<i>D. excentricus</i>					
TRIAL A		TRIAL B			
HIGH FOOD	LOW FOOD	HIGH FOOD	LOW FOOD		
<u>WH</u> (1)	<u>WH</u> (1)	WH (2)	<u>WH</u> (2)		<u>WH</u> (2)
		<u>WH</u> (2)	<u>WH</u> (2)		<u>WH</u> (2)

individuals. The addition of substrate from adult habitats has proven to be an effective cue for larval settlement and metamorphosis in other echinoid species (Highsmith, 1982; Highsmith and Emler, 1986). Containers were examined daily for newly settled juveniles and gravel substrate was replaced every four days during the course of normal water changes. Upon settlement, juveniles were collected from culture containers via mouth pipette and measured under 200 \times magnification using a compound microscope fitted with an ocular micrometer. Two disk diameters were recorded: the longest diameter and the diameter perpendicular to it. From these two measures, disk area was calculated using the formula for an ellipse. After measurement, juveniles were rinsed briefly in isotonic ammonium formate, freeze dried, and stored for later measurement of organic content.

2.2. *Strongylocentrotus purpuratus*

Adult *S. purpuratus* were obtained in January 2004 from Marine Research and Educational Products (Carlsbad, CA) and maintained in tanks with recirculating ASW at 12 °C with a salinity of 35 ppt. Techniques for spawning adults, collecting gametes and fertilizing eggs were identical to those described above for *A. punctulata*.

Embryos were separated into individual blastomeres at the two-cell stage using a modification of the methods of Harkey and Whiteley (1980). Within 1 min after fertilization, eggs were passed through a 60- μ m mesh in order to remove the FE. Results from previous trials confirmed that the FE became too stiff to be easily removed more than 1 min after rising. After the FE was removed, beakers containing the eggs were kept cool and monitored for signs of first cleavage. After approximately 30 min at 17 °C the embryos underwent first cleavage and were washed three times in an isosmotic solution of calcium and magnesium free seawater (CaMgFSW; see recipe in Strathmann, 1987). Embryos were left in CaMgFSW for a total time of less than 30 min. Extended exposure to CaMgFSW caused embryos to develop abnormally. After brief exposure to CaMgFSW the hyaline layer was dissolved and individual blastomeres were easily isolated from one another by gentle stirring. Embryos were then returned to normal ASW at 17 °C in order to continue normal development.

Following the blastomere separation procedure, embryos were sorted into 'whole' and 'half' size cultures by pouring through a 60 μ m mesh. Those embryos that had successfully been separated into individual blastomeres passed through this mesh, while those

that remained normal in size did not and were rinsed into a separate container. Two days following fertilization, embryos had developed into prism stage larvae and were sorted visually on the basis of size and placed in batches of 250 into 1 L culture containers. Two size classes were present per container for a total of 500 larvae L⁻¹. In each container one of the size classes was stained in order to distinguish between treatments. Staining and feeding protocols were identical to those described for *A. punctulata* (Table 1).

Unlike the other two species in this study, *S. purpuratus* larvae did not reliably metamorphose in the presence of substrate from adult habitat. Instead of settlement, age at metamorphosis was measured by determining the day on which the vestibule, an invagination of the ectoderm on the left side of the larval body, made contact with the left hydrocoel (Fig. 2). These two components will eventually form the rudiment that subsequently develops into the juvenile echinoid (Strathmann, 1987). The contact of the vestibule with the hydrocoel is an objective developmental landmark that can be used to compare relative rates of development across treatments, however this landmark is clearly an underestimate of the true time to metamorphosis and therefore only relative ages at 'metamorphosis' (e.g. between high and low food treatments) are discussed below. Because larvae settled sporadically, no comparisons of juvenile size were made across size or food treatments. However, measurements of disk diameter and organic content were made for the few individuals that successfully settled as juveniles.

2.3. *Dendraster excentricus*

Adult *D. excentricus* were collected from a large intertidal population in East Sound, Orcas Island, WA. Adults were collected in June 2001 and were immediately transported to Friday Harbor Laboratories, San Juan Island, WA where they were maintained at 12–15 °C in a flow through seawater system until August 2001. In August, adults were transported overnight to Chapel Hill, NC where they were maintained in a recirculating ASW system at 12 °C and 35 ppt. The same protocol was used in 2003 to collect and transport adult *D. excentricus*.

Protocols for spawning of adults, collection of gametes and fertilization of eggs were identical to those used for *A. punctulata*. Blastomeres were isolated at the two-cell stage using a modification of the methods described in Strathmann (1987). Within 5 min of fertilization, the FE and prominent jelly coat were removed by pouring the eggs through a 115 μ m mesh. This effectively removed

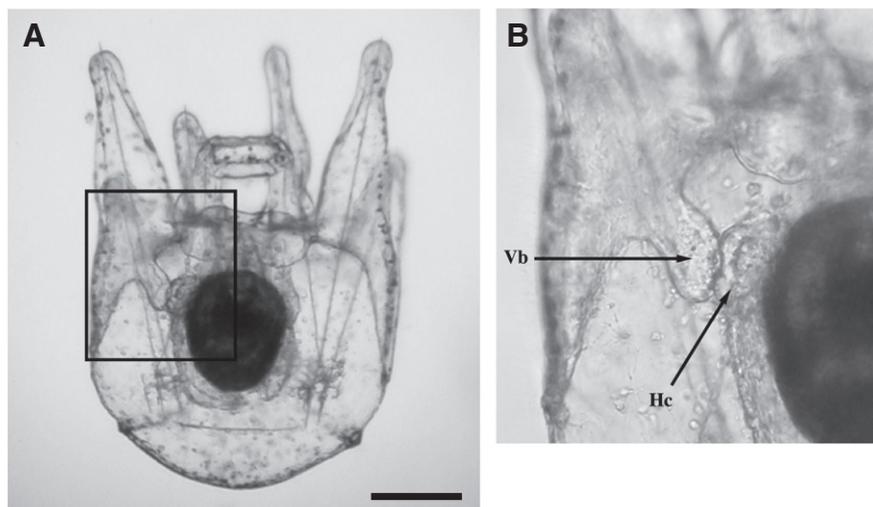


Fig. 1. Advanced pluteus larva of *S. purpuratus* showing initial signs of metamorphosis (A). Inset (B) shows magnified view of the vestibule (Vb) making contact with the hydrocoel (Hc). Scale bar represents 100 micrometers.

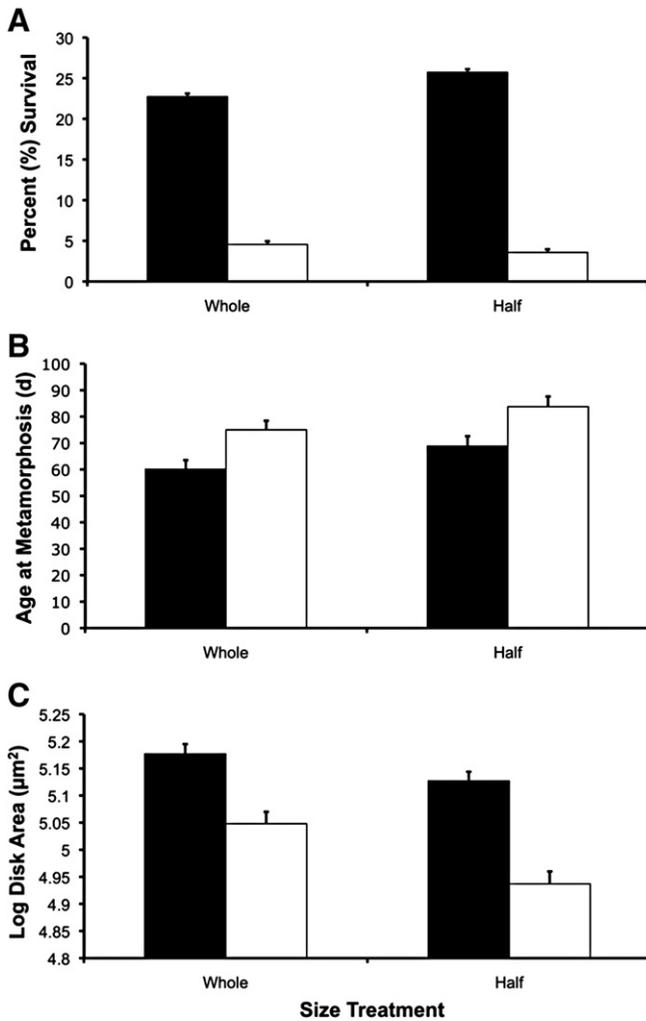


Fig. 2. *A. punctulata* average percent survival to metamorphosis (A), age at metamorphosis (B) and log disk area at settlement (C) for size treatments and food levels. Shaded bars are larvae from high food jars, unshaded bars are larvae from low food jars. In (A) bars are container means \pm SE. In (B) and (C) bars are estimated marginal means \pm SE from the mixed model ANOVA. Percent survival to metamorphosis was arcsin-square root transformed and disk area was log transformed before analysis to meet assumptions of normality.

the FE from approximately half of the embryos. All of the embryos were then washed three times in Calcium-free seawater (CaFSW; recipe in Strathmann, 1987) in order to dissolve the hyaline layer. Embryos remained in the final wash of CaFSW until first cleavage was complete, after approximately 1 h at 13 °C. Cleaved embryos were then poured through a 115 µm mesh in order to dissociate the individual blastomeres. Embryos that lost the FE during the first pass through the mesh were effectively isolated into individual blastomeres at this step. Those embryos that retained the envelope during the first pass remained as complete zygotes even after treatment with CaFSW and a second pass through the mesh. This method effectively produced large numbers of whole and half size embryos that were then returned to ASW and allowed to develop in environmental chambers at 13 °C.

One day after the blastomere separation procedure, embryos had reached the swimming blastula stage and were sorted into two size classes using a mouth pipet. Stain and food treatments were identical to those used for *A. punctulata* (Table 1). Larvae were reared through metamorphosis for two male–female pairs, one in 2001 and a second in 2003. When larvae became competent to settle, a small amount of sand from aquaria housing adults was added to larval cultures in

order to induce metamorphosis (Highsmith, 1982). At settlement juveniles were removed from cultures, two disk diameters were recorded, and they were preserved for organic content analysis using the methods described for *A. punctulata*.

2.4. Organic content

A standard spectrophotometric assay (Gosselin and Qian, 1999) was used to estimate the organic content of individual newly-metamorphosed juveniles. The protocol outlined by Gosselin and Qian (1999) was used with the following modifications: (1) a KI-starch solution was used as a substitute for Cdl-starch solution, and (2) the KI-starch solution was not filtered. Glucose standards were made in increments of 20, 15, 10, and 5 µg C by dilution with distilled water from a 40 µg C ml⁻¹ stock solution. Three replicates of each standard were used to create a standard curve on each day of measurement. Sample absorbance was converted to µg C using the standard curve.

2.5. Statistical analysis

Treatment effects were tested using the mixed models procedure of SPSS (version 12.0). For *A. punctulata* and *D. excentricus*, data were analyzed for the following dependent variables: percent survival through metamorphosis, age at metamorphosis, and initial disk area. SIZE, FOOD, STAIN, and the interaction between SIZE and FOOD were analyzed as fixed effects, and TRIAL and JAR as random effects. The fixed effect STAIN was dictated by the experimental design but was not of theoretical interest. In instances where STAIN was a significant effect, the interaction between STAIN and SIZE was also analyzed. Analyses include data from all trials unless specified. Initial disk area and age at metamorphosis were log-transformed, and percent survival was arc-sine square root transformed, in order to meet assumptions of normality. For *S. purpuratus*, mixed effects models were used to analyze the percent of larvae that had reached metamorphosis. SIZE, FOOD, and STAIN were modeled as fixed effects, JAR as a random effect and age of individuals (factor AGE) as a covariate.

In order to compare the results of the current study with previously published work, data from several studies were combined to test for correlations between initial egg size and the effects of egg size reductions on age and size at metamorphosis. Linear regression was used to test for significant relationships between initial egg size and the effects of egg size reductions on development time and size at metamorphosis. Initial egg volume was log transformed prior to applying linear regression analysis. To control for the potential effects of phylogeny, independent contrasts (Felsenstein, 1985; Harvey and Pagel, 1991) were estimated and data were reanalyzed, again using linear regression. The phylogenetic relationships among species were estimated based on the most recent phylogeny of echinoids available (Kroh and Smith, 2010).

3. Results

3.1. *A. punctulata*

The eggs of the *A. punctulata* females used in this study were 80.2 ± 1.7 µm in diameter ($N=2$ females; 10 eggs per female). Larvae from all combinations of size classes and food levels survived to metamorphosis. There was a significant effect of both FOOD and STAIN, but not of SIZE on the percent of *A. punctulata* larvae surviving to metamorphosis (Table 2A; Fig. 2A). Percent survival increased with FOOD but decreased with STAIN. FOOD and SIZE, as well as STAIN, had a significant effect on both age at metamorphosis (Table 2B; Fig. 2B) and size at metamorphosis (Table 2C; Fig. 2C). Age at metamorphosis decreased with increasing FOOD, SIZE, and STAIN and size at

Table 2

Mixed-Model ANOVA table for the dependent variables percent survival (A), age at metamorphosis (B), and disk area (C) for *A. punctulata*. Data for percent survival were arcsine square-root transformed, and data for disk area were log transformed prior to analysis to meet normality assumptions. JAR and TRIAL were included as random effects in each model. Significant effects ($P < 0.05$) are in bold.

Fixed effects	df	F	p
(A) SIZE	1, 17	0.107	0.748
FOOD	1, 16	28.760	<0.001
STAIN	1, 17	8.089	0.011
SIZE*FOOD	1, 17	4.104	0.059
(B) SIZE	1, 1531	41.677	<0.001
FOOD	1, 18	9.122	0.007
STAIN	1, 1522	6.103	0.014
SIZE*FOOD	1, 1531	0.001	0.986
(C) SIZE	1, 1536	47.438	<0.001
FOOD	1, 16	37.794	<0.001
STAIN	1, 1524	7.507	0.006
SIZE*FOOD	1, 1535	7.244	0.007

metamorphosis increased with FOOD and SIZE, but decreased with STAIN. There was also a significant interaction between SIZE and FOOD on size at metamorphosis (Table 2C). An interaction between STAIN and SIZE was tested but was not significant for any of the dependent variables ($P > 0.05$), and therefore that factor was removed from the final analyses. There was a significantly positive relationship between disk area and organic content at metamorphosis for juveniles from both whole ($R^2 = 0.1896$; $F_{1,115} = 26.904$, $P < 0.001$; Fig. 5A) and half ($R^2 = 0.2339$; $F_{1,15} = 4.580$, $P = 0.049$; Fig. 5A) SIZE treatments. Additionally, an analysis of covariance using disk area as the covariate revealed an effect of SIZE on organic content such that, for a given disk area, juveniles from half-size treatments had a higher organic content than juveniles from whole-size treatments ($F_{1,88} = 9.433$; $P = 0.003$).

3.2. *S. purpuratus*

The eggs of the *S. purpuratus* female used in this study were $87.5 \pm 0.9 \mu\text{m}$ in diameter ($N = 20$ eggs). The percentage of larvae undergoing metamorphosis increased significantly with SIZE, FOOD, and AGE but decreased significantly with STAIN (Table 3; Fig. 3). The interaction between STAIN and SIZE was not significant ($P > 0.05$) and so it was removed from this analysis. While few juveniles were reared to metamorphosis, there was a significantly positive relationship between disk area and the organic content of juveniles at metamorphosis ($R^2 = 0.1926$; $F_{1,21} = 5.008$; $P = 0.036$; Fig. 5B).

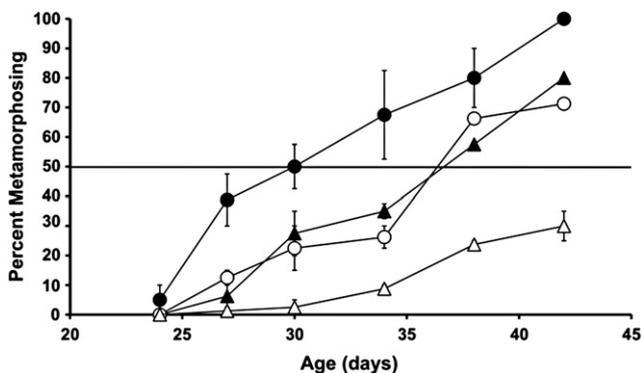


Fig. 3. Average percent of *S. purpuratus* larvae metamorphosing for each size treatment and food level. Circles represent whole size larvae. Triangles represent half size larvae. Filled symbols represent high food cultures and open symbols represent low food cultures. Bars are container means \pm SE. The horizontal line represents 50% metamorphosis.

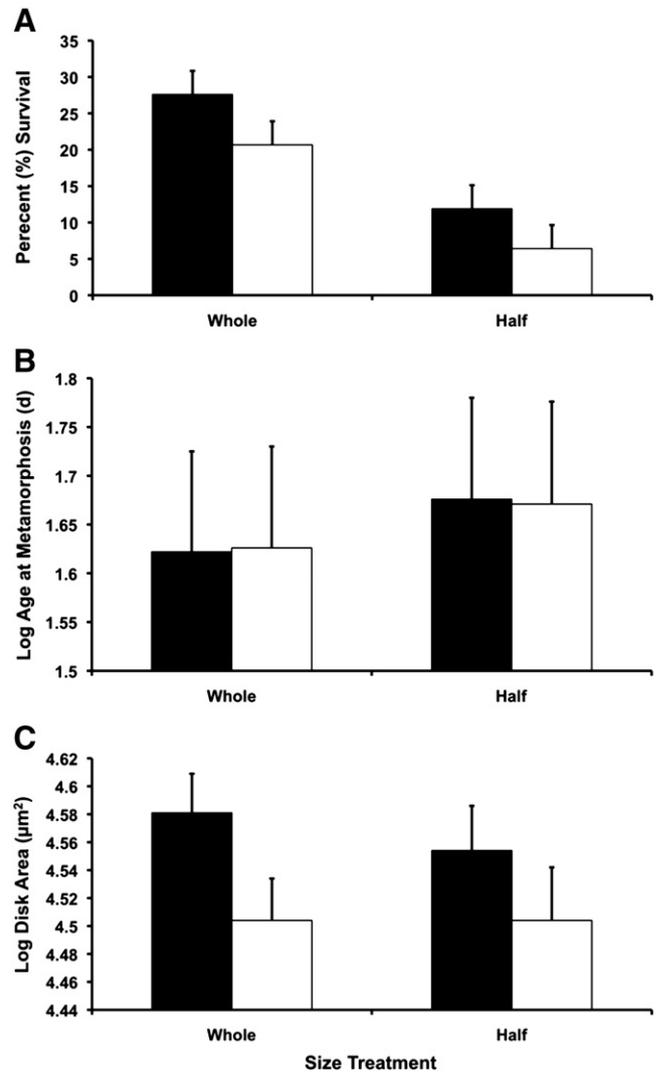


Fig. 4. *D. excentricus* average percent survival to metamorphosis (A), age at metamorphosis (B) and disk area at settlement (C) for size treatments and food levels. Shaded bars are larvae from high food jars, unshaded bars are larvae from low food jars. In (A) bars are container means \pm SE. In (B) and (C) bars are estimated marginal means \pm SE from the mixed model ANOVA. Percent survival to metamorphosis was arcsin-square root transformed and age at metamorphosis and disk area was log transformed before analysis to meet assumptions of normality.

Effects of SIZE, FOOD and STAIN on time to metamorphosis were estimated by a mixed model ANOVA on the time to which 50% of larvae exhibited contact of the vestibule and hydrocoel (Fig. 1). There was a significant effect of SIZE ($F_{1,6} = 74.128$; $P < 0.001$) and FOOD ($F_{1,6} = 66.939$; $P < 0.001$) but not of STAIN ($F_{1,5} = 1.644$; $P = 0.256$) on this measure of time to metamorphosis. There was also a significant interaction between SIZE and FOOD ($F_{1,5} = 7.550$; $P = 0.040$). Decreasing SIZE significantly increased time to metamorphosis while increasing FOOD significantly reduced time to metamorphosis. The significant interaction was due to an increased difference in time to metamorphosis between low and high food treatments for whole size embryos relative to half embryos.

3.3. *D. excentricus*

The eggs of the *D. excentricus* females used in this study were $124.2 \pm 0.4 \mu\text{m}$ in diameter ($N = 2$ females; 10 eggs per female). Percent survival to metamorphosis increased significantly with SIZE

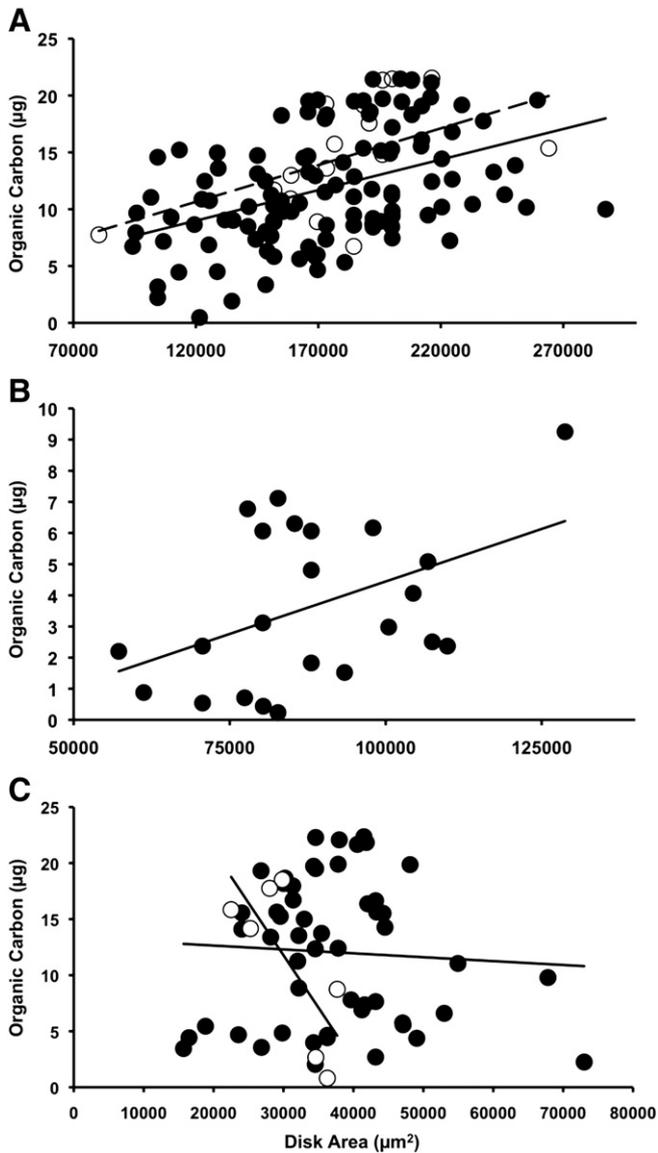


Fig. 5. Organic content versus disk area for new metamorphs of *A. punctulata* (A); *S. purpuratus* (B) and *D. excentricus* (C). In A, metamorphs are distinguished by initial egg size: whole (solid symbols, $N=111$; solid regression line, $R^2=0.1896$) and half (open symbols, $N=17$; dashed regression line, $R^2=0.2339$). In B, only whole size metamorphs are shown (B: $N=22$; $R^2=0.1926$). In C, metamorphs are distinguished by initial egg size as well: whole ($N=59$; $R^2=0.0034$) and half ($N=7$; $R^2=0.555$).

but FOOD and STAIN had no significant effects (Table 4A; Fig. 4A). Age at metamorphosis decreased significantly with increasing SIZE but was unaffected by FOOD or STAIN (Table 4B; Fig. 4B). Neither SIZE nor FOOD nor STAIN had a significant effect on size at metamorphosis (Table 4C; Fig. 4C). Finally, unlike the other two species in this study, there was no significant relationship found between disk area and organic content at metamorphosis (whole juveniles: $R^2=0.0034$;

Table 3
Mixed-Model ANCOVA table for the dependent variable *percent larvae metamorphosing* for *S. purpuratus*. JAR was included as a random effect, and age was used as a covariate.

Fixed effects	df	F	p
SIZE	1, 61	113.994	<0.001
FOOD	1, 6	49.640	<0.001
STAIN	1, 61	4.495	0.038
SIZE*FOOD	1, 61	2.214	0.142
AGE (Cov)	1, 62	285.055	<0.001

Table 4
Mixed-Model ANOVA table for the dependent variables *percent survival* (A), *age at metamorphosis* (B), and *disk area* (C) for *D. excentricus*. Data for percent survival were arcsine square-root transformed, and data for age at metamorphosis and disk area were log transformed prior to analysis to meet normality assumptions. JAR and TRIAL were included as random effects in each model. Significant effects ($P<0.05$) are in bold.

Fixed effects	df	F	P
(A) SIZE	1, 7	66.172	<0.001
FOOD	1, 7	1.525	0.257
STAIN	1, 7	0.402	0.546
SIZE*FOOD	1, 7	0.093	0.769
(B) SIZE	1, 616	15.674	<0.001
FOOD	1, 7	0.001	0.997
STAIN	1, 541	3.180	0.075
SIZE*FOOD	1, 618	0.178	0.673
(c) SIZE	1, 583	0.543	0.462
FOOD	1, 9	2.287	0.167
STAIN	1, 462	0.598	0.440
SIZE*FOOD	1, 597	0.755	0.385

$F_{1, 51} = 7.364$, $P = 0.677$; half juveniles: $R^2 = 0.555$; $F_{1, 5} = 27.864$, $P = 0.055$; Fig. 5C).

4. Discussion

All three of the species examined in this study exhibited significantly increased development times when offspring were reared from eggs whose volume had been reduced by 50%. These results confirm that for species of planktotrophic echinoids with small eggs (<130 µm), one cost of reduced egg size is a prolonged development time.

In addition to an increase in development time, another potential cost of small egg size is a reduction in juvenile size (Hart, 1995). In the current study, juvenile size was significantly reduced in *A. punctulata* but not *D. excentricus*. Juvenile size was significantly reduced in four previous studies on echinoids with experimentally reduced egg sizes (Alcorn and Allen, 2009; Allen et al., 2006; Emler and Hoegh-Guldberg, 1997; Hart, 1995). A fifth study found no significant effect of reductions in egg size on juvenile size (Sinervo and McEdward, 1988). As discussed further below, the inconsistent effects of egg size reductions on juvenile size may be due to the lack of a correlation between egg size and juvenile size in echinoids (Emler et al., 1987). Additionally, our poor understanding of echinoid juvenile ecology means that we do not have strong predictions about the proxies for performance that should be measured to estimate juvenile quality (e.g. are bigger juveniles necessarily better?) and therefore egg size may be strongly correlated with these unknown and unmeasured proxies (e.g. development of Aristotle's lantern, spine length, spine number, etc.).

The vital stain Nile Blue Sulfate was used in the current study so that embryos and larvae from the two size treatments could be reared together in the same container. Nile Blue Sulfate has been used in previous studies of echinoderm larvae with no detectable effects on development in some species (Allen et al., 2006; Simon, 1974), while in other species it has been shown to have both negative and positive effects on development (Alcorn and Allen, 2009). In the current study, the use of the stain significantly reduced percent metamorphosis but had no effect on development time in one species (*S. purpuratus*), decreased development time in a second species (*A. punctulata*), and had no effect on development time in a third (*D. excentricus*). One concern regarding the effect of the stain is that it might more negatively affect the halved embryos and therefore bias the results regarding the effect of size on development time and juvenile size. However, in no case was a significant interaction between size and stain found, suggesting that this potential bias was not present in the current study. In addition, when stain did have a significant effect on the dependent variable, this effect was generally much smaller than the effects of size or food treatment.

Thus, Nile Blue Sulfate is an effective vital stain for the developmental stages of echinoids, but future studies should be sure to include appropriate controls for its potential effects on development as these effects appear to vary across species.

4.1. The relationship between egg size and development time

Based on comparative data, evolutionary reductions in egg size are predicted to have exponentially increasing effects on development time as the initial size of the egg decreases (Levitan, 2000). However, the smallest egg sizes previously manipulated were 140 μm in *Echinarachnius parma* (Alcorn and Allen, 2009) and 155 μm in *Strongylocentrotus droebachiensis* (Alcorn and Allen, 2009; Hart, 1995; Sinervo and McEdward, 1988). For *S. droebachiensis*, Levitan's (2000) model predicts a 28% increase in development time after a 50% reduction in egg size. In contrast, the predicted increase in development time for the species with the smallest egg size used in the present study is 73% for *A. punctulata* (80 μm egg diameter).

In order to estimate the relationship between egg size and development time over a wide range of egg sizes, the results of the experimental manipulations in the current study were compared with those from four additional species (*Clypeaster rosaceus*: Allen et al., 2006; *Heliocidaris erythrogramma*: Emlet and Hoegh-Guldberg, 1997; *S. droebachiensis*: Alcorn and Allen, 2009; Hart, 1995; Sinervo and McEdward, 1988; and *Echinarachnius parma*: Alcorn and Allen, 2009). The average increase in development time resulting from a 50% reduction in egg size, expressed as a percent of total development time, was calculated for each of the seven species. Only experiments where food was not limiting (i.e. larvae were fed *D. tertiolecta* at a concentration of 2.5–5.0 cells μl^{-1}) were used for this comparison. For *S. droebachiensis*, the percent increase in development time was calculated as an average of the results of three studies: Sinervo and McEdward (1988), Hart (1995) and Alcorn and Allen (2009). Sinervo and McEdward (1988) did not report the exact increase in development time in their study, but this increase was estimated from Fig. 3 of their paper. Emlet and Hoegh-Guldberg (1997) were unable to detect any change in development time in *H. erythrogramma* after a 50% reduction in egg size, but the resolution of this study was low as the time over which metamorphosis was observed (1 day) was large relative to the short larval development period (3.5 d). A negligible increase in development time of 0.01% was assigned to this species in order to avoid using a non-zero number when fitting regressions. The increase in development time for all five species was then plotted against the initial size (volume calculated from measurements of diameter) of the egg and a linear regression was applied to the data following natural log transformation (Fig. 6A). In support of the fecundity-time hypothesis, there is a highly significant relationship between initial egg volume and the percent increase in development time following 50% reductions in egg volume ($R^2=0.769$; $P=0.009$). When the data in this analysis are plotted using independent contrasts to control for phylogenetic effects, the relationship does not change appreciably (Fig. 7A).

4.2. The relationship between egg size, juvenile size, and juvenile organic content

While comparative data support a negative correlation between egg size and development time in echinoids, the same data suggest that egg size is not correlated with size at metamorphosis (Emlet et al., 1987). In order to compare these data to the results from experimental manipulations of egg size, the relationship between egg size and juvenile size was plotted using the same methods described above for egg size and development time (Fig. 6B). As suggested by comparative data, there was no significant relationship between the effects of egg size on juvenile size across species

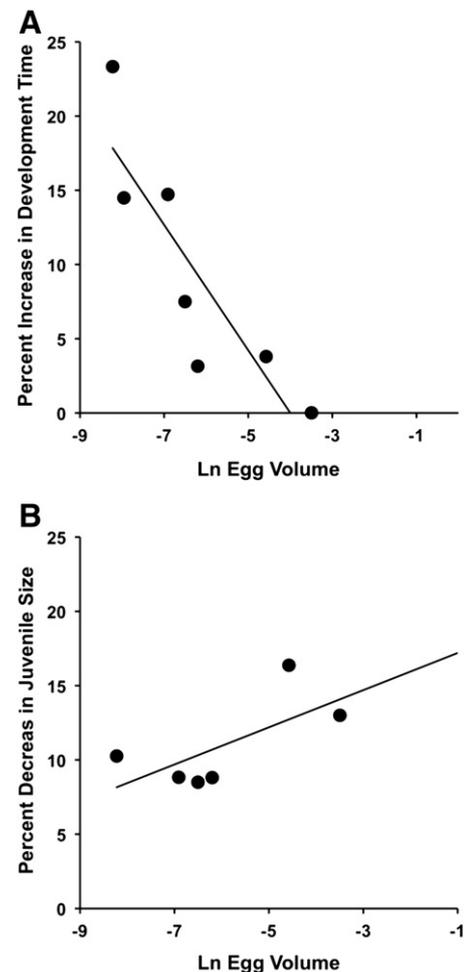


Fig. 6. Effects of 50% egg size reductions on echinoid echinoderms. The data in A and B represent the results from the three species in the current study along with the results of egg size reductions in *E. parma* (egg diameter = 142 μm ; Alcorn and Allen, 2009), *S. droebachiensis* (egg diameter = 155 μm ; Alcorn and Allen, 2009; Hart, 1995; Sinervo and McEdward, 1988), *C. rosaceus* (egg diameter = 270 μm ; Allen et al., 2006) and *H. erythrogramma* (egg diameter = 400 μm ; Emlet and Hoegh-Guldberg, 1997). Egg volume was ln transformed prior to regression analysis. The regression in (A) is described by the equation $y = -4.223x - 16.892$ ($R^2 = 0.769$; $P = 0.009$). The regression in (B) is described by the equation $y = 1.251x + 18.450$ ($R^2 = 0.458$; $P = 0.140$).

($R^2 = 0.458$; $P = 0.140$). In most cases, experimental manipulations within a species do result in significant decreases in juvenile size, but the magnitude of this effect does not appear to be correlated with the initial size of the egg. When the data in this analysis are plotted using independent contrasts, the relationship does not change significantly (Fig. 7B).

Recently, Pernet and Jaekle (2004) have shown that dichromate oxidation systematically underestimates protein levels in the eggs of marine invertebrates. Therefore, the organic content data presented here likely do not accurately reflect absolute energetic levels in juvenile echinoderms. However, even if these data consistently underestimate absolute levels of energy, it is likely that the relative energetic levels are accurately represented. In this study there was a significantly positive relationship between juvenile size and organic content in two of the three species examined. The relationship between juvenile size and organic content has been examined in only one other species of echinoid and a significantly positive relationship was also reported (Allen et al., 2006). The lack of a correlation between juvenile size and organic content in *D. excentricus* may also explain, in part, why no relationship was found between egg size and juvenile size in this species. If juvenile energetic content is independent of the size of the juvenile, then a reduction in

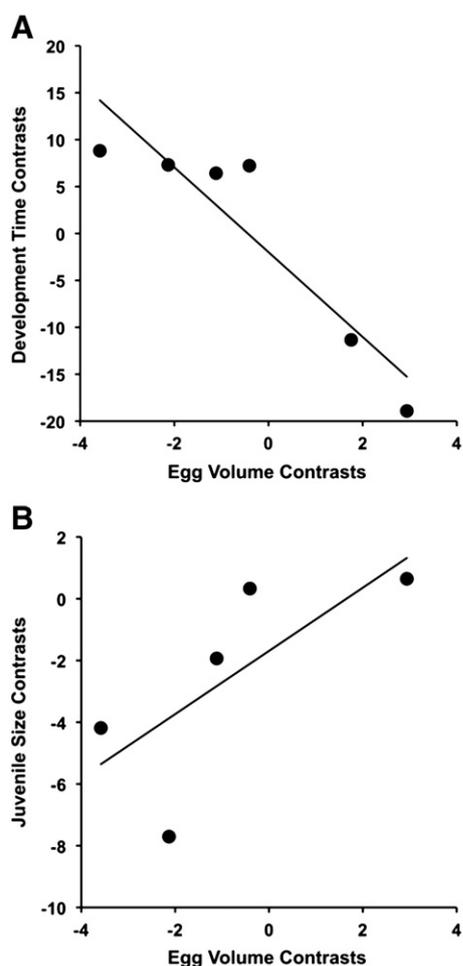


Fig. 7. Effects of 50% egg size reductions on echinoid echinoderms using independent contrasts. Data were ln transformed as in Fig. 6 and contrasts were estimated using the method of Felsenstein (1985). The regression in (A) is described by the equation $y = -4.518x - 1.998$ ($R^2 = 0.845$; $P = 0.01$). The regression in (B) is described by the equation $y = 1.024x - 1.690$ ($R^2 = 0.517$; $P = 0.171$).

juvenile quality caused by a reduction in egg size would not be detected through measures of juvenile size alone. However, when organic content was used as a metric of juvenile quality there was again no detectable difference between whole and half size juveniles. This suggests that the costs of egg size reductions in *D. excentricus* may be entirely compensated for by the increased time spent developing as larvae.

4.3. Other costs of small egg size

The current study examined only two of the potential costs of small egg size in marine invertebrates: increased development time and reduced juvenile size. Hart (1995) listed three other potential costs of small egg size: 1) lower fertilization rates, 2) increased predation rates, and 3) unsuitability for development in heterogeneous environments. The effect of egg size on fertilization rates is perhaps the best studied of the three alternative costs. Although smaller eggs do have lower fertilization rates (Levitan, 1993), recent evidence suggests that low fertilization rates exert relatively weak selective pressure on egg size (Podolsky, 2001) and that the addition of low-cost accessory structures (e.g. jelly coats) can circumvent this limitation of small egg size (Podolsky, 2004). The effects of egg size on predation rate are less well understood, but recent work (Allen, 2008) has shown that in many cases larvae developing from smaller eggs do indeed suffer enhanced rates of predation. However, when exposed to visual predators, smaller larvae are less likely to be

preyed upon than their large siblings or clonemates (Allen, 2008; Vaughn, 2010). Thus, increased mortality rates are likely to be a significant cost of small egg size under at least some, but not all, conditions. The effect of egg size on development in heterogeneous environments has only rarely been investigated in marine invertebrates, although as Hart (1995) points out it has been found to be a significant factor in determining optimal propagule size in amphibians (Crump, 1981), fish (Winemiller and Rose, 1993), and plants (Venable and Brown, 1988). Recent work in marine invertebrates suggests that bet-hedging strategies in development may be more common than currently appreciated and that intra-clutch variation in egg size can be one mechanism for generating such variation in developmental strategies (e.g. Krug, 2001, 2009).

While wide intraspecific variation in egg size has been known in echinoderms for some time (Turner and Lawrence, 1979), the suggestion that this variation may be adaptive as a bet hedging strategy is relatively new (Marshall et al., 2008). Demonstrating the functional relationship between offspring size and performance is therefore necessary to advance our understanding of the possibility of bet-hedging to evolve as a strategy in echinoderms. Here we have shown that for planktotrophic echinoids with small eggs, reductions in egg size significantly increase larval development time. In addition, in most species of echinoids, egg size reductions significantly reduce juvenile size. The extent to which egg size reductions influence these life-history parameters varies across species and, in the case of development time, is significantly correlated with the initial size of the egg. While egg size reductions likely have other deleterious consequences for the offspring of marine invertebrates, this study confirms the fecundity-time hypothesis as a potential explanation of the trade-off between egg size and development time in echinoderms.

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References

- Alcorn, N.J., Allen, J.D., 2009. How do changes in parental investment influence development in echinoid echinoderms? *Evol. Dev.* 11 (6), 719–727.
- Allen, J.D., 2008. Size-specific predation on marine invertebrate larvae. *Biol. Bull.* 214, 42–49.
- Allen, J.D., Zakas, C., Podolsky, R.D., 2006. Effects of egg size reduction and larval feeding on juvenile quality for a species with facultative-feeding development. *J. Exp. Mar. Biol. Ecol.* 331, 186–197.
- Cameron, R.A., Leahy, P.S., Davidson, E.H., 1996. Twins raised from separated blastomeres develop into sexually mature *Strongylocentrotus purpuratus*. *Dev. Biol.* 178, 514–519.
- Caswell, H., 1981. The evolution of “mixed” life histories in marine invertebrates and elsewhere. *Am. Nat.* 117 (4), 529–536.
- Christiansen, F.B., Fenchel, T.M., 1979. Evolution of marine invertebrate reproductive patterns. *Theor. Pop. Biol.* 16, 267–282.
- Crump, M.L., 1981. Variation in propagule size as a function of environmental uncertainty for tree frogs. *Am. Nat.* 117 (5), 724–737.
- Emler, R.B., 1995. Developmental mode and species geographic range in regular sea urchins (Echinodermata: Echinoidea). *Evolution* 49 (3), 476–489.
- Emler, R.B., Hoegh-Guldberg, O., 1997. Effects of egg size on postlarval performance: experimental evidence from a sea urchin. *Evolution* 51 (1), 141–152.
- Emler, R.B., McEdward, L.R., Strathmann, R.R., 1987. Echinoderm larval ecology viewed from the egg. In: Jangoux, M., Lawrence, J.M. (Eds.), *Echinoderm Studies*. Balkema, Rotterdam, pp. 55–136.
- Felsenstein, J., 1985. Phylogenies and the comparative method. *Am. Nat.* 125 (1), 1–15.
- Fox, C.W., 1997. Egg-size manipulations in the seed beetle *Stator limbatus*: consequences for progeny growth. *Can. J. Zool.* 75, 1465–1473.

- Gosselin, L.A., Qian, P.Y., 1999. Analysing energy content: a new micro-assay and an assessment of the applicability of acid dichromate assays. *Hydrobiologia* 390, 141–151.
- Harkey, M.A., Whiteley, A.H., 1980. Isolation, culture, and differentiation of echinoid primary mesenchyme cells. *Wilhelm Roux. Arch.* 189, 111–122.
- Hart, M.W., 1995. What are the costs of small egg size for a marine invertebrate with feeding planktonic larvae? *Am. Nat.* 146 (3), 415–426.
- Harvey, E.B., 1940. A new method of producing twins, triplets and quadruplets in *Arbacia punctulata*, and their development. *Biol. Bull.* 78, 202–216.
- Harvey, P.H., Pagel, M.D., 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford. 239 pp.
- Highsmith, R.C., 1982. Induced settlement and metamorphosis of sand dollar (*Dendraster excentricus*) larvae in predator-free sites: adult sand dollar beds. *Ecology* 63 (2), 329–337.
- Highsmith, R.C., Emler, R.B., 1986. Delayed metamorphosis: effect of growth and survival of juvenile sand dollars (Echinoidea: Clypeasteroidea). *Bull. Mar. Sci.* 39 (2), 347–361.
- Horstadius, S., 1973. *Experimental Embryology of Echinoderms*. Clarendon Press, Oxford. 192 pp.
- Kroh, A., Smith, A.B., 2010. The phylogeny and classification of post-Paleozoic echinoids. *J. Syst. Paleontol.* 8 (2), 147–212.
- Krug, P.J., 2001. Bet-hedging dispersal strategy of a specialist marine herbivore: a settlement dimorphism among sibling larvae of *Alderia modesta*. *Mar. Ecol. Prog. Ser.* 213, 177–192.
- Krug, P.J., 2009. Not my “type”: larval dispersal dimorphisms and bet-hedging in Opisthobranch life histories. *Biol. Bull.* 216, 355–372.
- Leviton, D.R., 1993. The importance of sperm limitation to the evolution of egg size in marine invertebrates. *Am. Nat.* 141 (4), 517–536.
- Leviton, 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. *Am. Nat.* 156 (2), 175–192.
- Marshall, D.J., Bonduriansky, R., Bussiere, L.F., 2008. Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. *Ecology* 89 (9), 2506–2517.
- McEdward, L.R., 1988. Experimental embryology as a tool for studying the evolution of echinoderm life histories. In: Paul, C.R.C., Smith, A.B. (Eds.), *Echinoderm Phylogeny and Evolutionary Biology*. Clarendon Press, Oxford, pp. 189–196.
- McEdward, L.R., 1996. Experimental manipulation of parental investment in echinoid echinoderms. *Am. Zool.* 36, 169–179.
- McEdward, L.R., 1997. Reproductive strategies of marine benthic invertebrates revisited: facultative feeding by planktotrophic larvae. *Am. Nat.* 150 (1), 48–72.
- Morgan, S.G., 1995. Life and death in the plankton: larval mortality and adaptation. In: McEdward, L.R. (Ed.), *Ecology of Marine Invertebrate Larvae*. CRC Press, Boca Raton, pp. 279–321.
- Nager, R.G., Monaghan, P., Houston, D.C., 2000. Within-clutch trade-offs between the number and quality of eggs: experimental manipulations in gulls. *Ecology* 81 (5), 1339–1350.
- Pernet, B., Jaekle, W.B., 2004. Size and organic content of eggs of marine annelids, and the underestimation of egg energy content by dichromate oxidation. *Biol. Bull.* 207, 67–71.
- Podolsky, R.D., 2001. Evolution of egg target size: an analysis of selection on correlated characters. *Evolution* 55 (12), 2470–2478.
- Podolsky, R.D., 2004. Life-history consequences of investment in free-spawned eggs and their accessory coats. *Am. Nat.* 163 (5), 735–753.
- Roff, D.A., 1992. *The Evolution of Life Histories: Theory and Analysis*. Chapman and Hall, London.
- Roff, D.A., 2002. *Life History Evolution*. Sinauer Associates, Sunderland, MA.
- Rumrill, S.S., 1990. Natural mortality of invertebrate larvae. *Ophelia* 32 (1–2), 163–198.
- Simon, K.A., 1974. Vital staining of *Patiria miniata* larvae. *Mar. Biol.* 25, 335–337.
- Sinervo, B., 1990. The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. *Evolution* 44 (2), 279–294.
- Sinervo, B., McEdward, L.R., 1988. Developmental consequences of an evolutionary change in egg size: an experimental test. *Evolution* 42 (5), 885–899.
- Smith, C.C., Fretwell, S.D., 1974. The optimal balance between size and number of offspring. *Am. Nat.* 108 (962), 499–506.
- Stearns, S.C., 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford.
- Strathmann, R.R., 1985. Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Annu. Rev. Ecol. Syst.* 16, 339–361.
- Strathmann, M.F., 1987. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. University of Washington Press, Seattle.
- Turner, R.L., Lawrence, J.M., 1979. Volume and composition of echinoderm eggs: implications for the use of egg size in life-history models. In: Stancyk, S.E. (Ed.), *Reproductive Ecology of Marine Invertebrates*. University of South Carolina Press, Columbia, pp. 25–40.
- Vance, R.R., 1973a. On reproductive strategies in marine benthic invertebrates. *Am. Nat.* 107 (955), 339–352.
- Vance, R.R., 1973b. More on reproductive strategies in marine benthic invertebrates. *Am. Nat.* 107 (955), 353–361.
- Vaughn, D., 2010. Why run and hide when you can divide? Evidence for larval cloning and reduced larval size as an adaptive inducible defense. *Mar. Biol.* 257, 1301–1312.
- Vaughn, D., Allen, J.D., 2010. The peril of the plankton. *Integr. Comp. Biol.* 50 (4), 552–570.
- Venable, D.L., Brown, J.S., 1988. The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. *Am. Nat.* 131 (3), 360–384.
- Winemiller, K.O., Rose, K.A., 1993. Why do most fish produce so many tiny offspring? *Am. Nat.* 142 (4), 585–603.
- Young, C.M., Chia, F.S., 1987. Abundance and distribution of pelagic larvae as influenced by predation, behavior and hydrographic factors. In: Giese, A.C., Pearse, J.S., Pearse, V.B. (Eds.), *Reproduction of Marine Invertebrates, Vol. 9: Seeking Unity in Diversity*. Blackwell/Boxwood Press, Palo Alto, pp. 385–463.