Effects of egg size reduction and larval feeding on juvenile quality for a species with facultative-feeding development

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Abstract

In free-spawning marine invertebrates, larval development typically proceeds by one of two modes: planktotrophy (obligate larval feeding) from small eggs or lecithotrophy (obligate non-feeding) from relatively large eggs. In a rare third developmental mode, facultative planktotrophy, larvae can feed, but do not require particulate food to complete metamorphosis. Facultative planktotrophy is thought to be an intermediate condition that results from an evolutionary increase in energy content in the small eggs of a planktotrophic ancestor. We tested whether an experimental reduction in egg size is sufficient to restore obligate planktotrophy from facultative planktotrophy and whether the two sources of larval nutrition (feeding and energy in the egg) differentially influence larval survival and juvenile quality. We predicted, based on its large egg size, that a reduction in egg size in the echinoid echinoderm *Clypeaster rosaceus* would affect juvenile size but not time to metamorphosis. We reduced the effective size of whole (W) zygotes by separating blastomeres at the two- or four-cell stages to create half- (H) or quarter-size (Q) "zygotes" and reared larvae to metamorphosis, both with and without particulate food. Larvae metamorphosed at approximately the same time regardless of food or egg size treatment. In contrast, juveniles that developed from W zygotes were significantly larger, had higher organic content and had longer and more numerous spines than juveniles from H or Q zygotes. Larvae from W, H and Q zygotes were able to reach metamorphosis without feeding, suggesting that the evolution of facultative planktotrophy in *C. rosaceus* was accompanied by more than a simple increase in egg size. In addition, our results suggest that resources lost by halving egg size have a larger effect on larval survival and juvenile quality than those lost by withholding particulate food.
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1. Introduction

In organisms that undergo development without parental care, the energy required for growth and development can be derived from two sources: parental investment in the egg and nutrient acquisition by offspring from the external environment. The degree of reliance on one or the other source defines a continuum of adult reproductive strategies whereby investment per egg is traded off against fecundity (Smith and Fretwell, 1974; Roff, 1992; Stearns, 1992). In marine invertebrates, energy for planktonic larval development can be derived from the egg, from consuming particulate food or from uptake of dissolved organic matter (Manahan, 1990; Jaeckle, 1995). In a subset of these species, energy reserves in the egg are sufficient to fuel larval development without additional nutrition (Thorson, 1950). However, the degree of offspring reliance on exogenous nutrition varies substantially across taxa,

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within taxa and even within individual clutches (McEdward and Carson, 1987; McEdward and Coulter, 1987; Eckert, 1995; Jaeckle, 1995; Herrera et al., 1996; George, 1999). In light of such variation, it is unclear whether changes in maternal provisioning and food supply have equivalent consequences for larval and juvenile success. Here we evaluate the relative importance of endogenous and exogenous resources to growth and survival during the larval and juvenile stages of a marine invertebrate.

The endogenous resources available to marine invertebrate larvae are reflected in the size of the egg. Across diverse marine invertebrate taxa, egg diameter is positively correlated with investment per offspring (Emlet et al., 1987; McEdward and Carson, 1987; Jaeckle, 1995), negatively correlated with fecundity (Thorson, 1950) and predictive of the mode of larval development (Thorson, 1950; Wray and Raff, 1991; Jeffery and Swalla, 1992). Larvae from species with small eggs are typically planktotrophic (obligately feeding) and require an extended period in the plankton, while those from large eggs are lecithotrophic (obligately non-feeding) and settle from the plankton relatively quickly. Because mortality in the plankton can be high (Young and Chia, 1987; Rumrill, 1990; Morgan, 1995), the benefits of a decrease in development time are thought to offset the cost to fecundity. These two developmental strategies are common among most groups of marine invertebrates (Levin and Bridges, 1995) and life-history models indicate that each strategy can be evolutionarily stable (Vance, 1973; Strathmann, 1985; Levitan, 2000).

When considering costs and benefits of the two strategies, models have traditionally focused on the period from fertilization to metamorphosis (e.g. Vance, 1973; McEdward, 1997; Levitan, 2000). The primary trade-off assumed by these models is that the gain in fecundity resulting from a reduction in egg size can offset the costs of increased time swimming and feeding in the plankton as a larva. However, some models and empirical data suggest that, as egg size increases, excess energy in the egg will have a diminishing effect on reducing development time (Emlet, 1995; Levitan, 2000). These observations suggest that investment in eggs could influence not only the length of the larval period, but also the quality of post-larval stages (Strathmann, 1977; McEdward and Janies, 1997; Pechenik, 1999).

Maternal investment in eggs can also influence the degree of dependence on exogenous food during development (Herrera et al., 1996). Although most species can be categorized as planktotrophs or lecithotrophs, a few species have an intermediate mode of development, facultative planktotrophy, where larvae can feed but do not require food to complete metamorphosis. Species with this rare mode of development provide an excellent experimental system for examining the developmental effects of both maternal investment and larval feeding. In particular, experimental manipulations of egg size and food availability can be used to separate their effects. In echinoderms, separation of blastomeres at the two-cell stage of development can produce half-volume "zygotes" that develop into fully formed larvae, which can complete metamorphosis (Driesch, 1892; Harvey, 1940; Horstadius, 1973) and become sexually mature adults (Cameron et al., 1996). Manipulations to reduce egg energy content increased the time to metamorphosis in planktotrophic species (Sinervo and McEdward, 1988; JDA, unpublished data), but reduced the size at metamorphosis in a lecithotroph (Emlet and Hoegh-Guldberg, 1997). These results reflect a difference in how endogenous resources are used by planktotrophic and lecithotrophic species, and suggest that juvenile quality could be improved by increasing maternal investment beyond a level sufficient for larvae to complete metamorphosis.

Manipulations of egg size in a species with facultative planktotrophy also offer a way to test hypotheses about the widespread evolutionary transition from feeding to non-feeding development (Herrera et al., 1996; McEdward, 1996). In particular, theory suggests that facultative planktotrophy is an evolutionarily unstable stage in the transition toward lecithotrophy that results from a simple increase in egg provisioning (Wray, 1996). At this stage, evolutionary reversals to obligate-feeding development would be possible under selection for reduced egg size (Herrera et al., 1996). Beyond this stage, reversals become less likely as the complex structures necessary for feeding are reduced or lost entirely (Strathmann, 1985; Wray, 1996). By reducing the eggs of a facultative planktotroph to a size equivalent to those produced by species with obligate planktotrophy, we asked whether a change in egg size alone is sufficient to restore the mode of development to the ancestral condition.

Here we examine the effects of maternal and feeding energy sources on larval and juvenile growth and survival in the sea biscuit *Clypeaster rosaceus* (Linnaeus), one of only three echinoderm species known to be facultative planktotrophs (Emlet, 1986; Hart, 1996; Allen and Podolsky, in review). By manipulating both egg organic content and food availability separately and in combination, we address the following questions: (1) Do larvae require food to complete metamorphosis

when egg volume is reduced to a size typical for obligate planktotrophy? (2) What are the consequences of reducing the availability of each source of energy for larval development and juvenile quality? (3) Are post-metamorphic measures of growth and survival more strongly affected by parental investment or by larval feeding?

2. Materials and methods

2.1. Egg size treatment

Adult *C. rosaceus* were obtained from the Keys Marine Lab, Florida and maintained at 23 °C in an aquarium with recirculating 35 ppt artificial seawater (ASW; Instant Ocean, Aquarium Systems Inc., Mentor, Ohio). Adults were induced to spawn gametes into ASW by vigorous shaking (Emlet, 1986). Eggs were then collected, washed once in clean ASW and fertilized by adding 1 ml of a dilute sperm solution. Egg diameters for unfertilized eggs of two of the females used were 273.5 ± 0.09 μm and 265.7 ± 0.35 μm (mean ± S.E.), similar to reports from previous studies (Emlet, 1986; Miner et al., 2002).

In order to generate offspring from half- (H) and quarter-size (Q) "eggs", blastomeres were separated at the two- or four-cell stages, respectively, to reduce the initial volume of whole (W) eggs (factor SIZE). For convenience, we refer to these three size classes as resulting from different egg sizes. For all three classes, the fertilization envelope (FE; 340-μm diameter) was removed approximately 3 min after fertilization by pouring eggs through a 280-μm mesh. Envelope-free zygotes were kept at 22 °C. When embryos had completed first cleavage (approximately 2 h post-fertilization), a portion were placed in calcium-free seawater (CaFSW) for 3 to 5 min to remove the hyaline layer (Strathmann, 1987). To create half-size (H) zygotes, the two sister blastomeres were then individually separated by repeated gentle pipetting up and down in a 50-μl capillary tube. These half-sized zygotes were returned to ASW and allowed to develop in an environmental chamber at 27–28 °C along with the remaining unseparated whole (W) zygotes. To create quarter-sized (Q) zygotes, the same method was used for a portion of embryos at the four-cell stage (approximately 1 h after first cleavage). Whole zygotes were also exposed to CaFSW for 3 to 5 min, but the dividing blastomeres were not pipetted apart. This allowed comparisons to be made among W, H and Q zygotes that had all received the same treatments to remove the FE and the hyaline layer. Prior to separation, the volumes

of the two or four divided blastomeres were not always equal. The unequal nature of cleavages may have contributed to variation within a size class that was not controlled for in our experiments.

Larvae were reared in 1000-ml tri-pour beakers, with two size classes per container. Previous studies have shown substantial container effects (Hart, 1996) and rearing (factor SIZE) classes together allowed us to reduce the number of containers. In feeding treatments (see below), food was maintained at a high enough level that we did not anticipate competition for food (Strathmann, 1971; Hart, 1991). Nevertheless, we varied which size classes were paired in order to control for and test the possibility that measurements depended on which particular size class was co-resident in the container (factor OTHER). Each container initially held 50 larvae from each of the two size classes.

In order to differentiate size classes within each container, one class per container was stained using the vital stain Nile Blue Sulfate (Allied Chemical Corporation; Simon, 1974). 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. Swimming blastulae (approximately 12 h old), when placed in the stain for approximately 20 min, turned noticeably blue and retained traces of stain as juveniles. The size treatment that was stained alternated between containers to control for possible effects of staining (factor STAIN). However, STAIN was not expected to affect development in *C. rosaceus*, because in three species of echinoids and one species of asteroid, Nile Blue Sulfate had no detectable effect on development (Simon, 1974; J.D. Allen, unpublished data). It was not possible to rear all three size classes in the same container simultaneously because a suitable second stain was not available at the time of the experiment and because equal numbers of all three size classes were not available for every cross.

2.2. Larval food treatment

Replicate containers were assigned at random to fed and unfed treatments (factor FOOD). Cultures of *Dunaliella tertiolecta* (UTEX Algal supply, Austin, Texas), a single-cell alga, were maintained in autoclaved ASW with modified Guillards f/2 medium (Florida Aqua Farms Inc.). Algae were separated from culture medium by brief centrifugation and were added to "fed" containers every other day at a concentration of 5 cells μl^{-1}. ASW was also filtered from containers and replaced every other day. Water in the containers was stirred by paddles at a rate of 10 strokes min^{-1} (Strathmann,

1987). In most cases, each SIZE × OTHER × STAIN × FOOD combination was replicated (Table 1).

Experiments were performed from crosses of three different male–female pairs (factor TRIAL). Size combinations used in each trial varied somewhat due to limitations on available numbers of half and quarter size eggs (Table 1).

2.3. Juvenile measurements

Substrate from adult habitat provides an effective cue for larval settlement and metamorphosis in echinoids (Highsmith, 1982; Emlet, 1986). Gravel from adult aquaria was added to containers once larvae became competent. Competence was determined by the presence of a large rudiment with visible spines within the larval body as well as by the shortening of larval arms. The most advanced larvae typically became competent after 10 days of larval development. Gravel substrate was replaced every 4 days during the course of normal water changes. Containers were completely surveyed daily under a dissecting microscope to record the date of metamorphosis for individual larvae. All larvae had either metamorphosed or died by 34 days post-fertilization.

On the day of metamorphosis, juveniles were collected from culture containers via mouth pipette and measured under 200× magnification using an ocular micrometer. Three measurements were recorded. *Disk area* was calculated from the formula for an ellipse, using disk diameter measured on the longest axis and the axis perpendicular to it. We also recorded total *spine number* and used the average length of the longest three spines to record *spine length*. Similar measurements have been used in previous experiments on echinoid juveniles (Emlet, 1986: Emlet and Hoegh-Guldberg, 1997) and, in a pilot study, we found noticeable treatment differences in each measurement. In order to follow individual survival and growth after initial measurement, each juvenile was placed into an individual

well of a six-well plate with 10 ml of ASW. Juveniles were not fed post-metamorphosis. We changed culture water and continued to measure disk diameters, spine length and spine number every 4 days until juvenile death. For post-metamorphic survival and growth, only juveniles from whole and half-size treatments were followed in sufficient numbers to report results. All juveniles had died by 40 days post-fertilization.

2.4. Organic content measurements

We used a standard spectrophotometric assay (Gosselin and Qian, 1999) to estimate the organic content of individual newly metamorphosed juveniles. At metamorphosis, juveniles were collected, measured as above, washed sufficiently in distilled water to remove residual chloride (Gosselin and Qian, 1999) and stored individually at −20 °C. We used the protocol outlined by Gosselin and Qian (1999) with the following modifications: (1) a KI-starch solution was used as a substitute for CdI-starch solution, (2) the KI-starch solution was not filtered and (3) the amount of each reagent used was quartered, which effectively reduced the assay resolution to a 0 to 5 μg C scale. Glucose standards were made in increments of 5, 2, 1.5, 1 and 0.5 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). We analyzed data for the following dependent variables: percent survival through metamorphosis, age at metamorphosis, initial disk area, initial spine number and initial spine length. SIZE, FOOD, STAIN, OTHER and the interaction between SIZE and FOOD were analyzed as fixed effects, and TRIAL and JAR as random effects. We compared four models, with and without each of the random terms, and selected the model that provided the best fit to the data using Akaike's Information Criterion (AIC) (Littell et al., 1996). Percent survival and age at metamorphosis were best modeled using both JAR and TRIAL as random effects. JAR alone provided the best fit in analyses of all other dependent variables. Two fixed effects–STAIN and OTHER–were dictated by the experimental design but were not of theoretical interest. When SIZE was a significant factor, post-hoc tests (sequential Bonferroni; Rice, 1989) were used to compare size classes. Analyses include data from all three

Table 1
Experimental design

Trial A		Trial B		Trial C	
Fed	Unfed	Fed	Unfed	Fed	Unfed
*W*H (2)	*W*H (2)	*W*H (2)	*W*H (2)	*W*H (1)	
W*H* (2)	W*H* (3)	W*H* (2)	W*H* (2)		W*H* (1)
	WQ (2)	*WQ* (2)	*WQ* (2)	*WQ* (1)	*WQ* (1)
	W*Q* (1)	W*Q* (2)	W*Q* (2)	W*Q* (1)	W*Q* (1)
			HQ (1)		
			H*Q* (1)	H*Q* (1)	H*Q* (1)

Size treatments are whole (W), half (H) and quarter (Q). Italicized letters are stained treatments. Number of replicate jars is in parentheses.

trials unless specified. Initial disk area and initial spine number were log-transformed and percent survival was arcsine-transformed in order to meet assumptions of normality.

To assess juvenile growth, in one trial we measured post-metamorphic changes in disk area, spine length and spine number. Absolute changes in disk area, spine length and spine number were calculated from measurements on days 0 and 4 after metamorphosis. Change between days 0 and 4 was used because growth in this period was typically greatest and most consistent across all trials; because juveniles were unfed, this interval provided the fairest comparison of the effects of egg size and larval feeding experience on initial juvenile growth. Mixed-effects models were used to test for the effects of FOOD, SIZE and their interaction on each of the three dependent variables at day 4, as well as the maximum disk area reached, with JAR treated as a random effect and the measure at day 0 used as a covariate. In addition, we analyzed for one trial the time from metamorphosis until death (juvenile age) and organic content at metamorphosis using mixed-effects models to test for the effects of FOOD, SIZE and their interaction, with JAR as a random effect. Initial disk area was used as a covariate in the analysis of organic content.

3. Results

Neither STAIN nor OTHER had a significant effect on any of the five variables measured at metamorphosis: disk area, spine length, spine number, percent survival or age at metamorphosis (Table 2). The low replication of the HQ combination (Table 1) relative to other size combinations lowered our power to detect the effects of OTHER. However, the high food levels and low larval densities (see Materials and methods) also made it unlikely that there was competition within a container. We concluded that neither of these aspects of experimental design influenced development and excluded STAIN and OTHER from the remaining analyses to maintain power for testing effects of interest.

3.1. Larval period

Larvae from each of the three SIZE classes–even those starting at one-quarter the normal egg volume–were able to reach metamorphosis without feeding. The percentage of larvae surviving to metamorphosis increased with size (Fig. 1A), but this effect was marginally non-significant ($F_{2,53}=2.975$, $P=0.06$). Because this test had directional predictions, we used pairwise comparisons to test for significant differences between

Table 2
Mixed-model ANOVA table for the dependent variables disk area (A), spine length (B) and spine number (C)

Fixed effects	df	F	P
(A) SIZE	2, 49	36.052	**<0.001**
FOOD	1, 40	4.235	**0.046**
STAIN	1, 913	0.232	0.630
OTHER	2, 43	0.341	0.713
SIZE * FOOD	2, 697	0.057	0.944
(B) SIZE	2, 49	2.152	0.127
FOOD	1, 38	6.927	**0.012**
STAIN	1, 860	2.413	0.121
OTHER	2, 43	0.389	0.680
SIZE * FOOD	2, 490	0.114	0.114
(C) SIZE	2, 50	10.276	**<0.001**
FOOD	1, 39	0.903	0.348
STAIN	1, 871	0.014	0.906
OTHER	2, 44	0.128	0.880
SIZE * FOOD	2, 512	2.836	0.060

Data for disk area and spine length were log-transformed prior to analysis to meet normality assumptions. JAR was included as a random effect in each model. Significant effects ($P<0.05$) are in bold.

each size class; however, none were found ($P>0.05$, sequential Bonferroni correction). FOOD did not have a significant effect on survival to metamorphosis ($F_{1,35}=0.031$, $P=0.861$; Fig. 1A).

All larvae that underwent metamorphosis did so within a period from 11 to 34 days after fertilization. Neither SIZE ($F_{2,42}=0.049$, $P=0.952$) nor FOOD ($F_{1,37}=0.844$, $P=0.364$) had a significant effect on time to metamorphosis (Fig. 1B).

3.2. Comparison of new metamorphs

Disk area at metamorphosis increased significantly with SIZE (Fig. 2A, Table 2) and was different for all pairwise comparisons of SIZE in the expected direction (sequential Bonferroni adjustment, $P<0.05$). Fed larvae had significantly larger disk areas at metamorphosis than unfed larvae (Fig. 2A, Table 2). The interaction between FOOD and SIZE was not significant (Table 2).

Spine length at metamorphosis was not affected significantly by SIZE (Fig. 2B, Table 2). In contrast, juveniles fed as larvae had significantly longer spines than those that were unfed (Fig. 2B, Table 2). The interaction between FOOD and SIZE was not significant (Table 2).

Spine number at metamorphosis differed significantly as a function of SIZE (Fig. 2C, Table 2), with all pairwise comparisons showing differences in the predicted direction (sequential Bonferroni adjustment, $P<0.05$). FOOD had no significant effect on initial
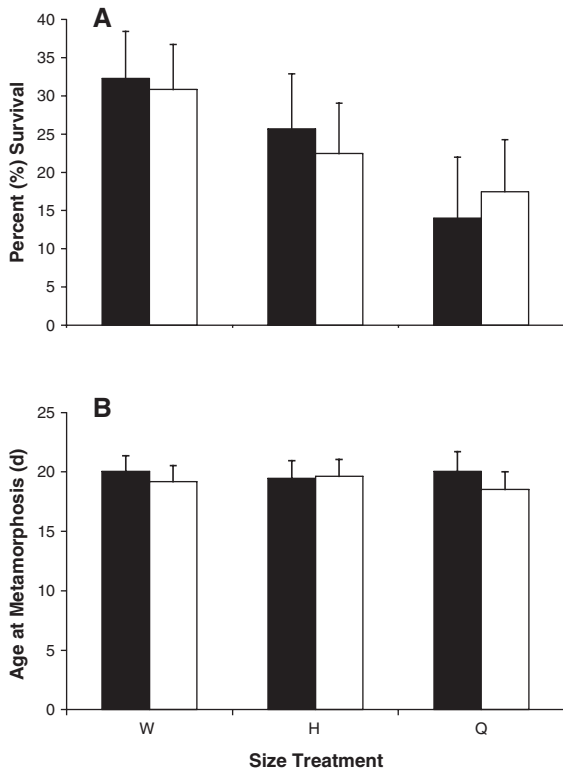
Fig. 1. Average percent survival to metamorphosis (A) and age at metamorphosis (B) for size treatments and food levels. Shaded bars are larvae from fed jars; unshaded bars are larvae from unfed jars. Bars are estimated marginal means ± S.E. from the mixed-model ANOVA. Percent survival to metamorphosis was arcsin-square root transformed before analysis to meet assumptions of normality.

spine number (Table 2) nor was there a significant interaction between FOOD and SIZE (Table 2).

A positive relationship was found between disk area and organic content at metamorphosis (Fig. 3). With disk area held constant, SIZE also contributed significantly to variation in the organic content per juvenile (Fig. 3A, Table 3). In contrast, FOOD did not have a significant effect on organic content when disk area was used as a covariate (Fig. 3B, Table 3). Thus, while disk area was an indicator of organic content ($R^2 = 0.3591$ for fed juveniles, $R^2 = 0.2832$ for unfed juveniles), juveniles of a given size that developed from larger eggs were of higher energetic quality than those that developed from smaller eggs, as seen in the elevation of the regression lines in Fig. 3A.

3.3. Post-metamorphic juvenile comparisons

Juvenile growth was greatest immediately after metamorphosis, continued through day 8, and then leveled off or declined (trial A, Fig. 4A–C). There

were no significant effects of SIZE or FOOD on either the absolute change in disk area (SIZE: $F_{1,98} = 0.891$, $P = 0.348$; FOOD: $F_{1,7} = 2.068$, $P = 0.192$) or relative change in disk area (SIZE: $F_{1,90} = 1.105$, $P = 0.296$; FOOD: $F_{1,7} = 3.229$, $P = 0.117$) between day 0 and day 4. There was, however, a significant effect of SIZE, but not of FOOD, on the absolute change in spine length (SIZE: $F_{1,85} = 12.572$, $P = 0.001$; FOOD: $F_{1,6} = 0.098$, $P = 0.764$) and the percent change in spine length (SIZE: $F_{1,137} = 9.145$, $P = 0.003$; FOOD: $F_{1,137} = 0.432$, $P = 0.512$). Neither SIZE nor FOOD had a significant
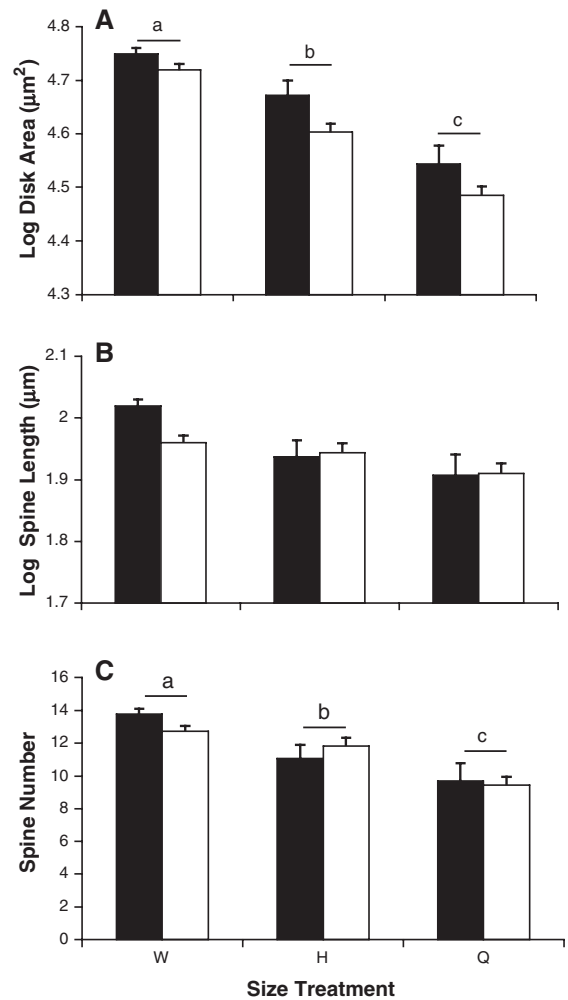

Fig. 2. Average disk area (A), spine length (B) and spine number (C) at metamorphosis for juveniles from whole, half and quarter size treatments across all trials. Shaded bars represent fed treatments; white bars indicate unfed treatments. Bars are estimated marginal means ± S.E. from mixed-model ANOVA. Letters over pairs of bars indicate significant differences between pairwise comparisons of size treatments based on sequential Bonferroni post-hoc tests. Disk area and spine length were each log-transformed in order to meet assumptions of normality.
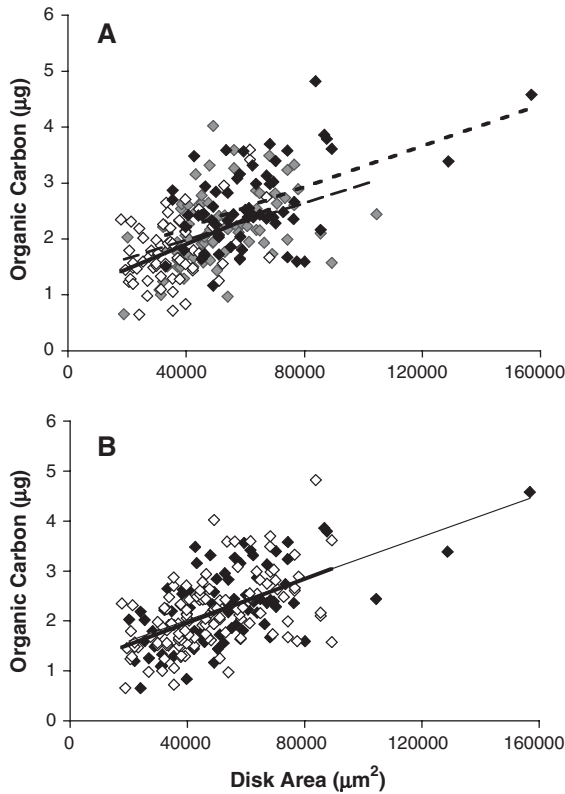
Fig. 3. Organic content versus disk area for new metamorphs. In A, metamorphs are distinguished by initial egg size: whole (solid symbols, $N=64$; short dashed regression lines, $R^2=0.2458$), half (gray symbols, $N=80$; long dashed regression line, $R^2=0.1639$) and quarter (open symbols $N=62$; solid regression line, $R^2=0.2035$). In B, metamorphs are distinguished by cultures that were fed (solid symbols, $N=84$; thick regression lines, $R^2=0.3591$) or unfed (open symbols, $N=122$; thin regression line, $R^2=0.2832$) as larvae. Metamorphs are from trial C only. Regression lines were fit to the data by treating each point independently.

effect on the absolute (SIZE: $F_{1,127}=0.070$, $P=0.792$; FOOD: $F_{1,10}=1.852$, $P=0.204$) or relative change in spine number (SIZE: $F_{1,112}=0.181$, $P=0.671$; FOOD: $F_{1,10}=1.263$, $P=0.289$).

There was a significant effect of SIZE, but not of FOOD, on the maximum disk area attained (Fig. 5A,

Table 3
Mixed-model ANCOVA table for the dependent variable *organic content* (total organic carbon)

Fixed effects	df	F	P
SIZE	2, 195	3.857	**0.023**
FOOD	1, 193	0.751	0.387
SIZE*FOOD	2, 196	2.083	0.127
Area (cov)	1, 197	47.048	**<0.001**

Analysis is for individuals in trial C only. JAR was included as a random effect and initial disk area (area) was used as a covariate.

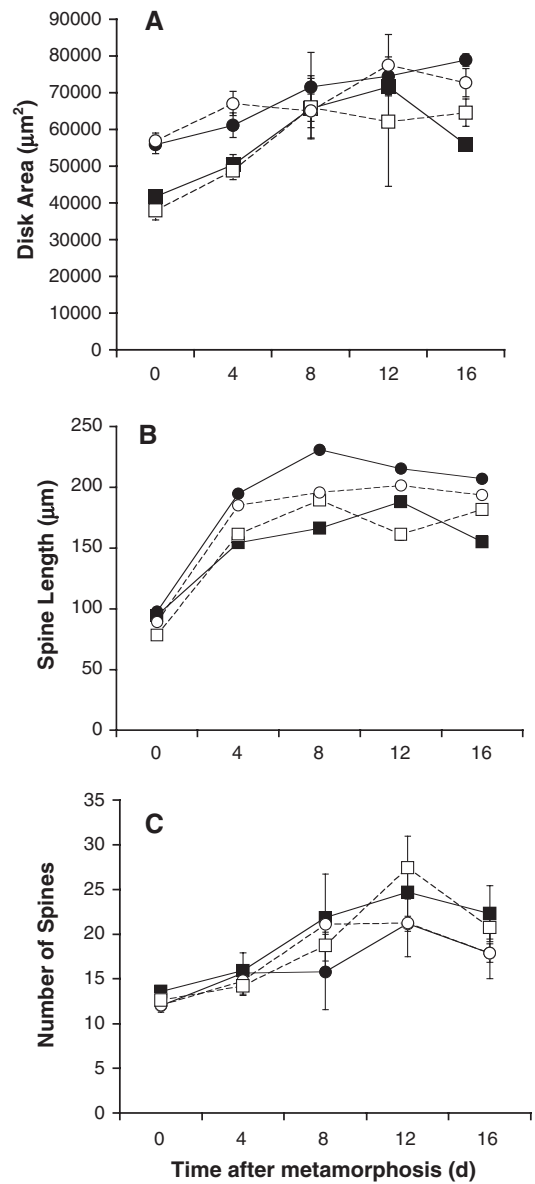

Fig. 4. Changes in juvenile disk area (A), spine length (B) and spine number (C) following metamorphosis. Points represent mean ± S.E. among containers for juveniles from whole eggs (circles) or half-size eggs (squares) that were fed (filled symbols, solid lines) or unfed (unfilled symbols, dashed lines). In B, error bars are smaller than data points. Juveniles are from trial A only.

Table 4). Juveniles from whole blastomeres reached a larger maximum size than did juveniles from half-size blastomeres. When initial disk area at metamorphosis was used as a covariate, FOOD but not SIZE had a significant effect on maximum disk area (SIZE: $F_{1,187}=0.041$, $P=0.840$; FOOD: $F_{1,187}=10.306$, $P=0.002$).

There was a significant effect of both SIZE and FOOD on the length of time that juveniles survived
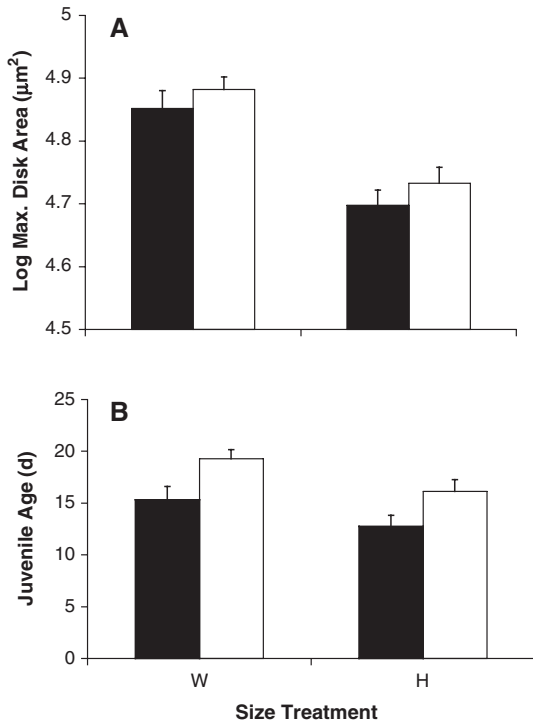
Fig. 5. Maximum juvenile disk area attained before death (A) and time from metamorphosis until death (B). Juveniles from whole and half size treatments were fed (shaded) or unfed (unshaded) as larvae. Juveniles are from trial A only. Bars are estimated marginal means ± S.E. from mixed-model ANOVA (Table 4). Maximum disk area was log-transformed prior to analysis.

following metamorphosis (AGE_D; Fig. 5B, Table 4). Juvenile time before death increased significantly with SIZE but, surprisingly, decreased significantly for juveniles fed as larvae.

4. Discussion

Manipulation of two energy sources used for development—maternal investment and particulate food—affected larval and juvenile growth and survival to different degrees. Egg size reduction generally had a larger effect on growth than did food availability. Egg size reduction significantly reduced juvenile disk area and spine number while food level had a significant effect only on juvenile spine length. We did not find significant interactions between egg size reductions and food level on larval or juvenile growth, suggesting that these two sources of nutrition are additive in their effects. Regardless of its source, the amount of larval energy available for development had a greater effect on size at metamorphosis than on time to metamorphosis. Following metamorphosis, egg size reduction had a

significant effect on the growth of spines, while larval food level did not significantly affect any measure of juvenile growth.

The relationship between egg organic content and egg size in *C. rosaceus* is of special importance given the use of its egg size in models of echinoid life-history evolution (McEdward, 1997; Levitan, 2000). In these models, the egg size of *C. rosaceus*–one of two echinoid species with facultative planktotrophy and the one with the smaller egg size–is assumed to be the minimum necessary for larvae to complete metamorphosis without feeding. Our results using halved and quartered eggs suggest that organic content of *C. rosaceus* eggs is not necessarily representative of this minimum. Instead, the eggs of *C. rosaceus* could represent the minimum size necessary to produce juveniles of sufficient size and energy to survive and grow in juvenile habitats. Models of the evolution of egg size should therefore consider the consequences of maternal investment beyond metamorphosis in order to predict conditions under which certain egg sizes would be favored (Strathmann, 1977). Current models, for example, may be overestimating the egg volume at which the primary effect of increases in investment shift from reducing larval development time to enhancing juvenile growth and survival.

In order to understand how increased energy content could be used to benefit offspring growth and development, it is necessary to understand when energetic reserves are being used. Our data provide an estimate of energy loss during development of a facultative planktotroph, as well as an estimate of the contribution of feeding to increasing energy content. Recent measures of organic content found that eggs of *C. rosaceus* contained 2.82 µg C (Miner et al., 2002). Assuming this starting value, we found that organic content declined by 18.5% to 25.5% in juveniles that were fed or unfed as larvae, respectively. In contrast, the lecithotrophic

Table 4

Mixed-model ANOVA table for the dependent variables *maximum disk area* (A) and *juvenile longevity* (B) for individuals from trial A only

Fixed effects	df	F	P
(A) SIZE	1, 153	52.142	**<0.001**
FOOD	1, 8	1.369	0.276
SIZE*FOOD	1, 153	0.018	0.894
(B) SIZE	1, 118	7.759	**0.006**
FOOD	1, 6	9.816	**0.021**
SIZE*FOOD	1, 118	0.078	0.780

Maximum disk area is the largest disk area attained by individual juveniles regardless of age. JAR was included as a random effect in each model.

echinoid *Heliocidaris erythrogramma* showed no loss of organic content during development (Hoegh-Guldberg and Emlet, 1997). Given some discrepancy in the literature concerning egg organic content measures (Miner et al., 2002; Emlet, 1986), however, measurements of egg and juvenile organic content within a single study will be needed to estimate absolute changes in organic content during the development of *C. rosaceus*.

4.1. Interaction between initial egg size and size reduction

For the few species where egg energy content has been manipulated, the effect of energy reduction appears to depend on initial egg size. For *C. rosaceus*, we found a 44% reduction in juvenile size when both egg size and food were maximally reduced, and no effect on time to metamorphosis. Similarly, Emlet and Hoegh-Guldberg (1997) found that, when lipids were removed by centrifugation from the large (400-μm diameter) eggs of the lecithotroph *H. erythrogramma*, juveniles metamorphosed smaller, grew less and died sooner than those from untreated eggs. In contrast, egg size reduction by blastomere separation for a planktotrophic species with smaller (152 μm) eggs, *Strongylocentrotus droebachiensis*, found either no change (Sinervo and McEdward, 1988) or only a minor decrease (Hart, 1995) in juvenile size; instead, this treatment increased the time to metamorphosis (Sinervo and McEdward, 1988). These experimental results, which are consistent with changes in development time among species that vary in egg energy content (Levitan, 2000), support the hypothesis that evolutionary increases in egg size should have a diminishing effect on larval period–and an increasingly large effect on juvenile quality–as egg size increases.

4.2. Benefits of feeding for a facultative planktotroph

In *C. rosaceus*, larval survival and time to metamorphosis were not significantly affected by food availability. Larval feeding did, however, significantly increase juvenile size at metamorphosis. These results are consistent with previous experiments on *C. rosaceus*, which found a significant effect of larval feeding on juvenile size but not on time to metamorphosis (Emlet, 1986). An obligately planktotrophic echinoid, *S. droebachiensis*, also produced larger juveniles at higher food levels (Hart, 1995). Similarly, in the gastropod *Crepidula fornicata*, juveniles from starved larvae had slower growth than those from fed larvae (Pechenik et al., 2002) and, in

the mussel *Mytilus galloprovincialis*, fed larvae were larger at metamorphosis and had larger organic reserves (Phillips, 2002). These differences in juvenile size between fed and unfed larvae suggest that feeding can provide a benefit to both facultative and obligate planktotrophs in terms of increased juvenile quality.

The difference in juvenile size between fed and unfed larvae, however, was much smaller than differences among blastomere size treatments. What explains the apparently greater influence of endogenous energy sources? First, maternal provisioning and feeding could supply different absolute quantities of energy. In order to evaluate this hypothesis one could estimate the amount of energy gained from larval feeding in *C. rosaceus*. Alternatively, feeding and maternal provisioning could provide qualitatively different nutritional substrates for larval development, such that one source is used in a way that more directly impacts growth. In echinoderms, for example, evolutionary increases in egg size have been accompanied by a shift toward greater egg lipid concentrations (Jaeckle, 1995). Furthermore, the lipid reserves of lecithotrophs, which include wax esters, appear to be used for post-metamorphic development. In contrast, juveniles from planktotrophic species do not show significant levels of wax esters at metamorphosis (Villinski et al., 2002). This difference between the composition of lecithotrophic juveniles and planktotrophic juveniles implies that evolutionary increases in maternal provisioning lead to a qualitative change in nutrition that differs from what is gained through larval feeding alone.

4.3. Juvenile growth and survival

In the period just following metamorphosis, larger juveniles did not grow in size or produce spines at a faster rate than smaller juveniles. This result is consistent with studies of the lecithotroph *H. erythrogramma* (Emlet and Hoegh-Guldberg, 1997) but not with studies of other marine invertebrates. For example, larger hatchlings of the gastropod *Nucella ostrina* had a faster absolute growth rate than smaller hatchlings from the same clutch (Moran and Emlet, 2001) and, in *M. galloprovincialis*, growth rate was also positively correlated with juvenile size (Phillips, 2002). Emlet (1986) hypothesized that larger juveniles of *C. rosaceus* would have higher growth rates than smaller juveniles because in other marine invertebrates growth rates are proportional to body size (Yamaguchi, 1975, 1977). Because we did not feed juveniles and prolong the period of juvenile growth, we could not test Emlet's (1986) prediction.

Although egg size and food did not influence post-metamorphic changes in disk size and spine number, egg size was positively related to relative and absolute growth rates of spines. Spine growth could be important to the survivorship of juvenile echinoids; Highsmith (1982) found that juveniles reach a size threshold (test diameter plus spine length) beyond which they become less vulnerable to sand bed predators. In addition, we found a significant positive relationship between egg size and juvenile lifespan (time from metamorphosis until death). Our results are similar to those for *H. erythrogramma*, which showed a positive relationship between juvenile lifespan and initial organic content (Emlet and Hoegh-Guldberg, 1997), and those for *N. ostrina*, where larger (higher organic content) juveniles survived longer (Moran and Emlet, 2001). These studies support the idea that energy acquired during the larval stage can be carried over into the juvenile stage and influence juvenile performance and survivorship (Pechenik et al., 1998; Marshall et al., 2003).

4.4. Facultative planktotrophy and the transition to non-feeding development

Life-history models have proposed that the first step in the evolutionary loss of larval feeding is an increase in energy investment per offspring, which releases larvae from dependence on food (Wray, 1996). Wray (1996) and McEdward (1996) suggested that one corollary of this hypothesis could be tested experimentally in a facultative feeder such as *C. rosaceus*: if an increase in energy content were the only change involved in the shift to facultative planktotrophy, then a reduction in maternal investment should restore dependence on food. Alternatively, if changes in investment were accompanied by other developmental changes, then a size reduction could lead to complete but slower development or to smaller juvenile size. Our finding that even quarter-size zygotes can reach metamorphosis, though at smaller size, is consistent with the second hypothesis. This result indicates that, if an obligately planktotrophic ancestor of *C. rosaceus* had eggs that were at least one-quarter its size, then the shift to facultative planktotrophy was accompanied by changes that permit metamorphosis at a smaller size.

One change that could have accompanied the evolution of large egg size in *C. rosaceus* is an increase in endogenous production of thyroid hormones. Recent work has shown that addition of thyroid hormone to cultures of larval echinoids can accelerate larval development and result in settlement at smaller sizes (Heyland et al., 2004; Heyland and Hodin, 2004). Thus, release from dependence on food, the primary source of thyroid hormone, could lead to evolutionary changes in endogenous hormone production that drives metamorphosis even when the maternal source of larval nutrition is artificially reduced (Heyland and Hodin, 2004).

The benefits of increased larval energy supply for post-larval development have important implications for the evolution of large egg size. Post-larval stages have not been considered in some models of marine invertebrate life-history evolution (Vance, 1973; McEdward, 1997; Levitan, 2000), but including post-larval consequences could help to explain the wide variation of egg sizes found in nature. Species that produce larger eggs than necessary to reach metamorphosis presumably suffer a fecundity cost (Vance, 1973; Strathmann, 1985; McEdward, 1997; Levitan, 2000), suggesting there must be additional benefits to maternal investment (Pechenik, 1999), some of which we identified in this study. By allowing extensive manipulation of two sources of energy for larval development, facultative planktotrophs provide an instructive model for understanding effects of maternal investment and larval feeding on performance across early life-history stages.

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