

Egg size and the evolution of phenotypic plasticity in larvae of the echinoid genus *Strongylocentrotus*

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Abstract

Planktotrophic larvae grow by utilizing energy obtained from food gathered in the plankton. Morphological plasticity of feeding structures has been demonstrated in multiple phyla, in which food-limited larvae increase feeding structure size to increase feeding rates. However, before larvae can feed exogenously they depend largely on material contained within the egg to build larval structures and to fuel larval metabolism. Thus, the capacity for plasticity of feeding structures early in development may depend on egg size. Using the congeneric sea urchins *Strongylocentrotus franciscanus* and *S. purpuratus*, which differ in egg volume by 5-fold, I tested whether the degree of expression of feeding structure (larval arm length) plasticity is correlated with differences in the size of the egg. I experimentally manipulated egg size of *S. franciscanus* (the larger-egged species) by separating blastomeres at the 2-cell stage to produce half-sized larvae. I reared half-size and normal-size larvae under high and low food treatments for 20 days. I measured arm and body lengths at multiple ages during development and calculated the degree of plasticity expressed by larvae from all treatments. Control and unmanipulated *S. franciscanus* larvae (from ~1.0 nl eggs) had significantly longer arms relative to body size and a significantly greater degree of plasticity than half-sized *S. franciscanus* larvae (from <0.18 nl eggs), which in turn expressed a significantly greater degree of plasticity than *S. purpuratus* larvae (from ~0.3 nl eggs). These results indicate that egg size affects larval arm length plasticity in the genus *Strongylocentrotus*; larger eggs produce more-plastic larvae both in an experimental and a comparative context. However, changes in egg size alone are not sufficient to account for evolved differences in the pattern of plasticity expressed by each species over time and may not be sufficient for the evolutionary transition from feeding to non-feeding. © 2007 Elsevier B.V. All rights reserved.

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1. Introduction

During larval development, organisms typically encounter unpredictable feeding environments (Conover, 1968). Consequently, planktonic larvae have the potential to be food-limited (Olson and Olson, 1989) and

experience high rates of mortality due to the indirect effect of a prolonged period that exposes larvae to greater levels of predation (Rumrill, 1990). Because of these harsh circumstances, there is strong selection for traits that ameliorate the effects of adverse feeding conditions (Doughty, 2002). Selection can act on phenotypic variation in traits associated with the utilization of two different energetic resources available to the larva: 1) the endogenous energetic reserves obtained from the parent or 2) the exogenous food resources acquired from the larval feeding environment.

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The degree of parental investment in an individual offspring is reflected in the size of the egg from which that individual develops (Jaekle, 1995). Egg size is correlated with initial size of the larva, larval habitat, the duration and rate of larval development, and the mode of larval nutrition (McEdward, 1986a, b). Egg size can affect an individual offspring's fitness (Vance, 1973; Christiansen and Fenchel, 1979; Strathmann, 1985; Sinervo and McEdward, 1988; Hart, 1995; reviewed by Havenhand, 1995; McEdward, 1996; Emler and Hoegh-Guldberg, 1997) and consequently is an important trait in life-history studies of many organisms (Emler et al., 1987). Egg size is a trait that can change in response to selection (Lessios, 1990; Jackson and Herrera, 1999; Moran, 2004). However, closely related species that inhabit the same larval environment, and likely experience similar selective pressures for resource acquisition, can have very different egg sizes (Wray and Raff, 1991; Herrera et al., 1996; Allen and Podolsky, 2007) suggesting that egg size may respond indirectly to selection on other species-specific life-history characteristics.

Phenotypic plasticity allows organisms to match trait expression to environmental heterogeneity (West-Eberhard, 2003). Morphological phenotypic plasticity in response to food resource level has been demonstrated in planktotrophic (feeding) larvae from multiple species in different phyla (Echinoderms: Boidron-Metairon, 1988; Hart and Scheibling, 1988; Strathmann et al., 1992, 1993; Fenaux et al., 1994; George, 1994, 1999; Hart and Strathmann, 1994; Sewell et al., 2004; Miner, 2005; Podolsky and McAlister, 2005; Reitzel and Heyland, 2007; Molluscs: Klinzing and Pechenik, 2000). Under low food availability, larvae increase the length of a food-collecting ciliated band, a response that is correlated with lengthening of skeletal arm rods in pluteus larvae. Longer arms increase the rate at which larvae clear food from suspension (Hart and Strathmann, 1994) and could increase the uptake of dissolved organic matter by changing larval surface area (Boidron-Metairon, 1988). For these reasons, arm length has been used as an indicator of larval nutritional history in the field (Strathmann et al., 1992).

Plasticity of larval feeding structures hinges on an energetic investment trade-off between larval and juvenile structures; increased investment in arms can result in decreased or delayed investment in other structures, such as the juvenile rudiment (Strathmann et al., 1992; Heyland and Hodin, 2004). Plasticity of arm length is expressed during early larval development (approximately 1 to 2 weeks post-fertilization for most species: see Echinoderm references listed above), suggesting that planktotrophic larvae may utilize endogenous resources for the

initial production of food-collecting structures, then move to exogenous resources for the development of other, later-appearing structures. Endogenous resources are provided to individual offspring within the egg and egg size is positively correlated with the level of investment (Jaekle, 1995). The capacity for plasticity of arm length early in development may therefore depend on the amount of maternally provisioned energetic reserves, and thus on egg size (Herrera et al., 1996).

There are two alternative hypotheses of the effect of egg size on plasticity. The first is an argument based on energy and materials in the egg: larvae from larger eggs may have a greater capacity for the expression of plasticity because they have access to and make use of a larger store of endogenous energy and materials. The second is an evolutionary argument: larvae that develop from smaller eggs may have been selected for a greater scope for plasticity to take better advantage of scarce exogenous food. Herrera et al. (1996) predicted that plasticity may be more important, but more difficult to express, in larvae that develop from smaller eggs. One recent study (Podolsky and McAlister, 2005) found support for this prediction among ophiuroid pluteus larvae. Ophiuroids possess a pluteus larval form that is similar in structure and function to the echinoid pluteus larva, and is thought to have evolved independently. Their study was not an explicit test of the hypotheses presented here; however, the authors found that smaller-egged species in the genus *Macrophiothrix* expressed plasticity of larval arm length, whereas larger-egged species did not. Another recent study (Reitzel and Heyland, 2007) specifically tested for an effect of egg size on the expression of phenotypic plasticity in echinoid pluteus larvae. Using subtropical irregular echinoid species from multiple genera, the results also indicate that plasticity of larval arm length was exhibited by smaller-egged species and not by larger-egged species.

Both Podolsky and McAlister's (2005) and Reitzel and Heyland's (2007) studies provide solid comparative datasets of plastic responses to decreased food levels in species (and genera) that develop from differently sized eggs, although Reitzel and Heyland's (2007) approach does not control for phylogeny or for the different environments that their species have evolved in. My study complements the results of these previous studies because I investigated within a species the effects of experimental manipulations of egg size on the expression of plasticity. By physically manipulating egg size, I am able to separate the two arguments (energy/materials and evolutionary) for why egg size may play a role in the expression of plasticity of feeding structures. In addition, I control for phylogeny and evolutionary environment by comparing

plastic expression between two species in the same genus that co-occur in the same habitat.

In this study, I investigated whether egg size affects the expression of larval arm length plasticity by experimentally halving egg size of *Strongylocentrotus franciscanus*. I experimentally reduced the amount of available endogenous material available to developing larvae, using blastomere separation at the 2-cell stage to produce viable offspring that were one-half normal-size (Driesch, 1892; Okazaki and Dan, 1954; Horstadius, 1973). This protocol provides a rigorous within-species test of the effect of egg size on the expression of plasticity. If *S. franciscanus* larvae from half-size eggs have a decreased capacity for plasticity early in development compared to *S. franciscanus* larvae from normal eggs, this would support the hypothesis that the amount of endogenous material in the egg can affect morphological plasticity. Alternatively, if larvae from half-size eggs show no difference in the capacity for plasticity early in development compared to larvae from normal eggs, this would support the hypothesis that egg size is linked to plasticity not through direct effects of the amount of material in the egg, but indirectly through the effects of natural selection acting simultaneously on multiple life-history characteristics.

In addition, I investigated the expression of larval arm length plasticity between species by examining larval development in two congeneric sea urchins in the genus *Strongylocentrotus* that have substantially different egg sizes. Adult animals in this genus are found in temperate coastal habitats off the Pacific coast of North America, have similar morphology and ecology, and develop via planktotrophic pluteus larvae (McEdward, 1986a, b; Strathmann, 1987). I examined larval growth and morphological plasticity in *S. franciscanus* and *S. purpuratus*, which co-occur and have egg diameters (volumes) of roughly 135 (1.29) and 80 (0.27) μm (nl), respectively (Emlet et al., 1987). I used these two species to elucidate the role of egg size on the evolved capacity for plasticity in this genus because they inhabit the same larval feeding environment and yet have naturally occurring variation in egg size.

2. Materials and methods

Adult *S. franciscanus* and *S. purpuratus* sea urchins were collected from a sub-tidal population located off the coast of Carlsbad, CA, by employees of Marine Research and Educational Products (M-REP, Inc.) in February 2006. The urchins were packed in moist paper towels and shipped overnight to Chapel Hill, NC, where they were maintained in a recirculating artificial seawater aquarium held at 15 °C and 33.5‰ salinity.

2.1. Larval culture

Upon receipt, adult urchins were induced to spawn gametes by peristomial injection into the body cavity of approximately 1 ml of 0.5 M KCl. Eggs were collected and washed once in artificial seawater (ASW: Instant Ocean, Aquarium Systems; 33.5‰ salinity), and sperm were collected by mouth pipette and kept on ice until use. Larval cultures of *S. franciscanus* were established by fertilizing eggs from 2 females with sperm from 5 males. Larval cultures of *S. purpuratus* were established by fertilizing eggs from 2 females with sperm from 7 males. Initial mean (\pm 1 SE) egg diameters (means of 10 eggs each) for the two *S. franciscanus* females were $122.7 \pm 0.35 \mu\text{m}$ and $124.7 \pm 0.17 \mu\text{m}$ and for the two *S. purpuratus* females were $81.8 \pm 0.00 \mu\text{m}$ and $85.2 \pm 1.12 \mu\text{m}$. Egg volumes (assuming a sphere) were 0.967, 1.015, 0.287, and 0.324 nl respectively.

Fertilized embryos and larvae were reared in one of two replicated food environments (5 and 0.5 algal cells/ μl). Each food level was then replicated among either three (*S. franciscanus*) or four (*S. purpuratus*) cultures. Each larval culture was fed the unicellular alga *Dunaliella tertiolecta* (UTEX Algal Supply, Austin, TX) daily, starting at 48 h (all ages reported are post-fertilization). All cultures were reared in ASW in 1-l plastic tri-pour beakers at densities of 1 larva ml^{-1} and water was changed every other day. The cultures were maintained in an environmental chamber held at 17 °C and were continually stirred at approximately 10 strokes min^{-1} with acrylic paddles to homogenize food and to keep larvae in suspension (Strathmann, 1987). *D. tertiolecta* was cultured at room temperature in autoclaved ASW enriched with a modified Guillard's f/2 medium (Florida Aqua Farms, Inc.). Algae were separated from the growth medium by centrifugation and then re-suspended in fresh ASW before use.

2.2. Blastomere separation

Blastomere separation at the 2-cell stage produces viable offspring that are one-half normal-size (Okazaki and Dan, 1954). Individual blastomeres were isolated at the 2-cell stage from a sub-set of the fertilized *S. franciscanus* embryos using a modification of a common *S. purpuratus* blastomere separation protocol (Harkey and Whiteley, 1980; Allen, 2005). To remove the fertilization envelope (FE), eggs were passed repeatedly through a 100 μm nitex mesh within 1 min post-fertilization. Upon removal of the FE, fertilized eggs were kept cool in glass dishes of ASW held on ice and monitored for signs of cleavage. The glass dishes were coated with a thin layer of 2% agar in ASW to prevent the fertilized eggs from sticking to the sides. After

approximately 2 h in chilled ASW, embryos underwent first cleavage and were washed 4 times with an isosmotic solution of calcium- and magnesium-free seawater (CaMgFSW; recipe in Strathmann, 1987). Brief exposure (less than 30 min) to CaMgFSW dissolved the hyaline layer; blastomeres were easily separated upon gentle stirring. Embryos were returned to chilled ASW after separation to continue development.

The blastomere separation protocol routinely produces two different size classes of embryos: ‘half’ size embryos that develop from dissociated blastomeres and ‘whole’ size embryos that develop from non-dissociated blastomeres. Following the separation protocol, embryos were sorted into whole- and half-size classes by pouring through a 70 μm nitex mesh. Half-size embryos passed through the mesh and whole-size embryos did not. In addition to the larval cultures established for larvae developing from untreated, ‘full’ size eggs (detailed above), larval cultures of whole- and half-size *S. franciscanus* were established from the embryos subjected to the blastomere separation protocol. Fertilized whole- and half-size embryos and larvae were reared in one of two different replicated food environments. Each food level was then replicated among three (whole-size *S. franciscanus*) or four (half-size *S. franciscanus*) cultures. Cultures of larvae that were subjected to the blastomere separation protocol were reared in the same manner as previously described for normally developing, untreated, full-size *S. franciscanus* and *S. purpuratus* larvae. The only difference in culture set-up was that half-size larvae were reared in smaller plastic tri-pour beakers (400 ml

instead of 1l), albeit at the same density as larvae in the other treatments.

2.3. Measures of phenotype

On days 3, 5, 7, 10, 13, and 16, approximately 10 larvae were removed from each culture. *S. purpuratus* larvae were also removed on day 20. The larvae were placed on a glass slide, immobilized with a dilute (<10%) solution of buffered formalin in ASW, and covered with a glass cover slip raised on clay feet. Three-dimensional Cartesian coordinates were recorded of multiple morphological features for 5 larvae from each culture (Fig. 1). These landmarks included the tip and base of each anterolateral and postoral arm rod, the posterior tip of the larva, the tip of the oral hood (i.e. the mid-point of the soft-tissue that stretches between the pair of anterolateral arms), and points at the anterior and posterior ends of the stomach. To collect data from each larva, I used a digitizing tablet (Hyperpen 12000U, Aiptek Inc.) to capture x and y coordinates of morphological landmarks. Simultaneously, I obtained z coordinates from a rotary encoder (U.S. Digital) coupled to the fine focus knob of a Wild M-20 compound microscope (McEdward, 1985). Using these 3-D Cartesian coordinates, I geometrically reconstructed individual arm, body, and stomach lengths for each larva. Because the postoral and anterolateral arms were the most prominent arms at the stages when I collected measurements, my analysis focuses on plasticity in their summed length (“total arm length”).

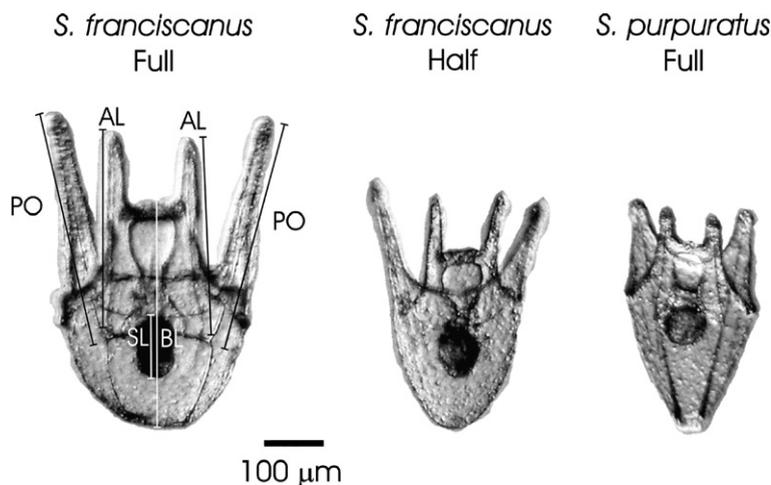


Fig. 1. Low-fed *Strongylocentrotus franciscanus* larvae from full- and half-size eggs and low-fed *S. purpuratus* larvae from a full-size egg. All larvae are from day 10 of development post-fertilization. Morphological characters that I measured on days 3, 5, 7, 10, 13, 16, and 20 (*S. purpuratus* only): AL = anterolateral arm, PO = postoral arm, BL = body length at midline, SL = stomach length. All larvae are displayed at the same magnification; scale bar represents 100 μm .

2.4. Statistical analysis

Two analysis of variance (PROC MIXED: SAS Institute, Cary, NC) tests were conducted using the results obtained in this study. For the comparison among normally developing (full-size eggs), treatment control (whole-size eggs), and treatment (half-size eggs) *S. franciscanus* larvae, I tested for the effect of variation among treatment, day of development (day), food level (food), and culture replicate on total arm length. The statistical model included terms to account for variation due to the interactions of treatment with food, treatment with day, day with food, and the three-way interaction of treatment by day by food. Treatment, food, day, and the interaction terms were coded as fixed effects and culture as a random effect. Day was coded as a repeated measure with culture as the subject; the type of covariance structure of the R matrix was specified as Compound Symmetry (CS). The factor culture was nested within treatment and food. Degrees of freedom were calculated using the DDFM = BW (Between-Within) option in PROC MIXED. Body length was included in the model as a quantitative covariate. I compared models both with and without the body length interaction terms and used the model (no interaction terms) that provided the better fit to the data using Akaike's information criteria (AIC) (Littell et al., 1996). The specific comparisons of effects due to treatment and treatment with food interaction between larvae developing from half- versus whole-, half- versus normal-, and normal- versus whole-size eggs were tested using the CONTRAST statement in PROC MIXED (SAS Institute, Cary, NC). For the comparison between normally developing (full-size eggs) *S. franciscanus* larvae with normally developing *S. purpuratus* larvae, I tested for the effect of variation among species (instead of treatment) and all of the factors described above. The 'treatment' and 'species' models were the same except for the terms used to account for the effects due to either treatment or species and their interactions with the other factors (the interaction terms). Arm and body length values for individual larvae were natural log-transformed prior to analysis for both statistical tests to meet the assumptions of normality.

To investigate differences in the degree of plasticity expressed by individuals from each species and/or treatment over time, I calculated the absolute and percentage differences in mean total arm length between food treatments on each measurement day. I also calculated these values for mean relative arm length (arm length: body length ratio). Positive deviations from zero indicate that low-fed larvae had longer arms, either absolutely or relative to body length, than high-fed larvae. Lastly, I

calculated the average percent difference across days in relative arm length expressed by larvae within each species and treatment.

3. Results

Modification of the blastomere separation procedure used for *S. purpuratus* larvae by Allen (2005) was successful; separation of *S. franciscanus* blastomeres at the two cell stage produced embryos and larvae that were approximately half-sized (Fig. 1). However, yield of half-size larvae was low, necessitating the use of smaller beakers for larval culture. There were no half-size larvae available for measurement after day 13.

ANOVA among full-, whole-, and half-size *S. franciscanus* larvae detected significant effects of treatment, day, food, and the interactions of treatment with day, day with food, and the three-way interaction of treatment by day by food (Table 1). ANOVA also detected a significant effect due to body length. There was no effect due to the interaction of treatment with food. The results for the specific contrasts are also presented in Table 1: the specific contrasts of a treatment effect between half-versus whole- and half- versus full-size larvae were significant, indicating that larvae from full- and whole-size eggs developed longer arms when controlling for body size than larvae from half-size eggs. The specific

Table 1
Analysis of variance (ANOVA) results for full- versus whole- versus half-sized *Strongylocentrotus franciscanus* larvae

	df	F value	Pr > F
<i>Source</i>			
Treatment	2, 14	25.99	<0.0001
Day	4, 44	246.45	<0.0001
Food	1, 14	80.96	<0.0001
Treatment * day	8, 44	9.24	<0.0001
Treatment * food	2, 14	0.46	0.6418
Day * food	4, 44	10.92	<0.0001
Treatment * day * food	8, 44	2.68	0.0171
Body length	1, 389	510.63	<0.0001
<i>Contrasts: treatment</i>			
Half vs. whole	1, 14	36.26	<0.0001
Half vs. full	1, 14	46.99	<0.0001
Full vs. whole	1, 14	1.32	0.2704
<i>Contrasts: treatment by food</i>			
Half vs. whole	1, 14	0.38	0.5450
Half vs. full	1, 14	0.88	0.3639
Full vs. whole	1, 14	0.10	0.7533

Dependent variable is total arm length with body length as a quantitative covariate. Listed also are the results of the specific contrasts of an effect due to treatment and to treatment by food interaction between whole-versus half-size larvae.

Table 2
Analysis of variance (ANOVA) results for full-size *Strongylocentrotus franciscanus* versus full-size *S. purpuratus*

Effect	df	F value	Pr>F
Species	1, 10	1352.13	<0.0001
Day	6, 51	87.67	<0.0001
Food	1, 10	73.20	<0.0001
Species * day	5, 51	26.80	<0.0001
Species * food	1, 10	20.06	0.0012
Day * food	6, 51	4.11	0.0020
Species * day * food	5, 51	1.36	0.2545
Body length	1, 397	498.89	<0.0001

Dependent variable is total arm length with body length as a quantitative covariate.

contrast of a treatment effect between full- and whole-size larvae was not significant. The specific contrasts of a treatment by food interaction effect between half- versus whole-, half- versus full-, and full- versus whole-size larvae were not significant, indicating that there was no difference in the effect of the interaction of treatment with food (i.e. the degree of plasticity) on the expression of arm length when controlling for body size. ANOVA between

untreated full-size *S. franciscanus* and *S. purpuratus* larvae detected significant effects of body length, species, day, food, and the interactions of species with day, species with food, and day with food (Table 2). There was no significant effect of the three-way interaction of species by day by food.

Calculation of the mean percent difference between low- and high-fed larvae across all days within each treatment indicated that low-fed larvae had arms that were absolutely longer than high-fed larvae (see Fig. 2 and Table 3): full-size *S. franciscanus* (mean 5.51%; range -6.81% to 15.41%); whole-size *S. franciscanus* (mean 10.12%; range -7.13% to 20.40%); half-size *S. franciscanus* (mean 13.25%; range -0.70% to 22.91%); full-size *S. purpuratus* (mean 2.34%; range -3.37% to 5.58%). A similar calculation indicated that low-fed larvae had arms that were longer relative to body size than high-fed larvae: full-size *S. franciscanus* (mean 11.88%; range 2.64% to 17.45%); whole-size *S. franciscanus* (mean 10.37%; range 0.45% to 16.05%); half-size *S. franciscanus* (mean 8.40%; range -0.28% to 15.46%); full-size *S. purpuratus* (mean 4.53%; range 0.19% to 9.77%). High-fed larvae

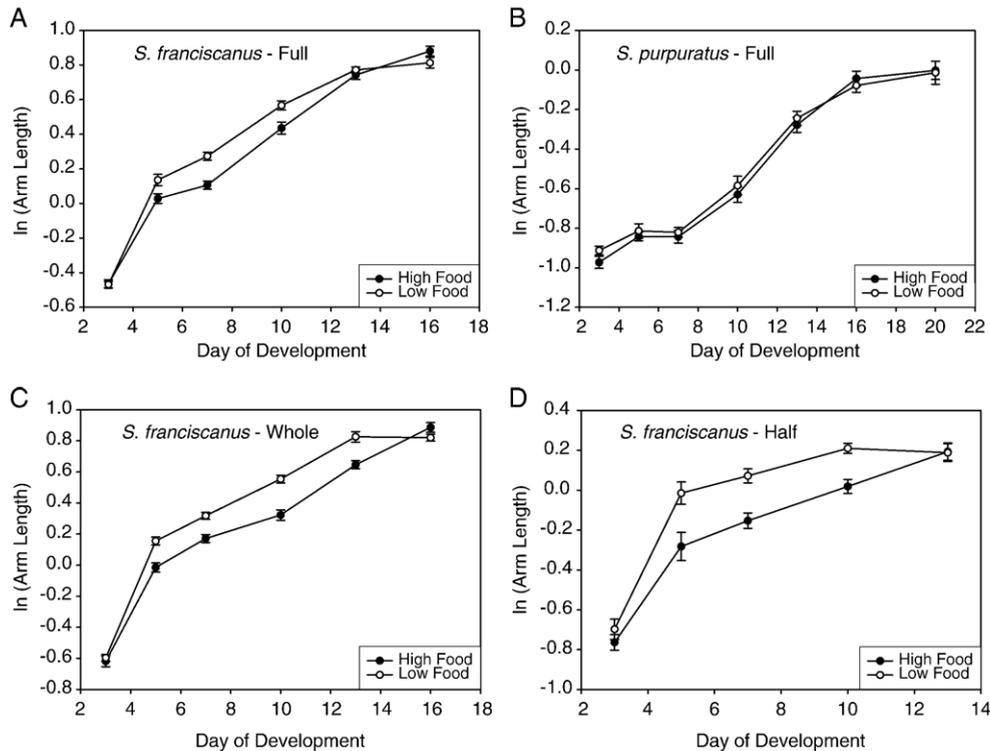


Fig. 2. Mean summed length of postoral and anterolateral arms (\pm 1SE) for high-fed (filled symbols) and low-fed (open symbols) larvae over time. Arm length values for individual larvae were natural log-transformed before means were calculated. A) *Strongylocentrotus franciscanus* larvae from full-size eggs. B) *S. purpuratus* larvae from full-size eggs. C) *S. franciscanus* larvae from whole-size eggs: individual blastomeres did not dissociate during the blastomere separation treatment. D) *S. franciscanus* larvae from half-size eggs: individual blastomeres dissociated into half-size “eggs” during the blastomere separation treatment.

Table 3

Mean percent difference in absolute total arm length (normal text) and relative arm length (total arm to body length ratio: bold text) by species, treatment, and day

Day	<i>S. franciscanus</i>		<i>S. purpuratus</i>	
	Full	Whole	Half	Full
3	-0.54	1.65	6.83	5.43
	2.64	0.45	1.25	1.11
5	10.39	15.51	22.91	3.57
	8.47	11.31	11.65	0.19
7	15.41	13.53	20.11	1.82
	15.04	6.43	13.93	6.91
10	11.98	20.40	17.08	5.58
	15.43	16.05	15.46	2.85
13	2.61	16.73	-0.70	3.52
	17.45	15.42	-0.28	9.77
16	-6.81	-7.13		-3.37
	12.24	12.54		6.76
20				-0.16
				4.12

Positive values indicate that low-fed larvae had either absolutely or relatively longer arms than high-fed larvae. Note: values of arm and body length were not natural log-transformed for this analysis.

had arms that were longer relative to body size than low-fed larvae (negative value) only on day 13 for half-size *S. franciscanus* (Table 3).

Differences in degree of plasticity of arm:body length ratios (using natural log-transformed values) among treatments over time are depicted graphically in Fig. 4. Positive deviations from zero indicate low-fed larvae had longer arms, relative to body length, than high-fed larvae. *S. franciscanus* larvae from the full-, whole-, and half-size treatments all exhibited a similar pattern in the trajectory of their degree of plasticity curves: there was no significant effect due to the interaction of treatment with food (Table 1). Degree of plasticity increased rapidly through day 10 (excluding day 7 for the whole-size treatment) and plateaus at approximately 0.15 (all treatments) before decreasing slowly by day 16 (full- and whole-size) or decreasing rapidly to zero (half-size) by day 13. Considered collectively, the pattern exhibited by the trajectories of the *S. franciscanus* treatments differ from the pattern exhibited by *S. purpuratus*: there was a significant effect due to the interaction of species with food (Table 2). For *S. purpuratus*, degree of plasticity increased slowly over time, fluctuating with each measurement around approximately 0.05, before decreasing slowly after day 13.

4. Discussion

In the Introduction, I have suggested two hypotheses regarding the effect of egg size on plasticity. The first

was an energy/materials argument: larvae from larger eggs may have a greater scope for the expression of plasticity because they have access to and make use of the materials in a larger egg. The second was an evolutionary argument: larvae that develop from smaller eggs may have been selected for a greater capacity for plasticity to energetically discount their earlier dependence on exogenous food. The results presented here suggest that for *Strongylocentrotus*, the role of egg size in plasticity may derive from a combination of both hypotheses.

First, in response to low food, whole-size *S. franciscanus* larvae had relatively longer arms than half-size *S. franciscanus* larvae. The results of the contrast statements in the ANOVA indicate a significant difference between these two treatments (Table 1). Similarly, averaged over time, the mean percent difference in relative arm length between low- and high-fed larvae was 10.4% for whole- compared to 8.4% for half-size larvae. These results support the energy/materials argument because halving egg size via blastomere separation decreased the degree of plasticity that larvae expressed.

Second, in response to low food, untreated full-size *S. franciscanus* larvae (egg diameters from two adult females: $122.7 \pm 0.35 \mu\text{m}$ and $124.7 \pm 0.17 \mu\text{m}$) had relatively longer arms than untreated full-size *S. purpuratus* larvae (egg diameters from two adult females: $81.8 \pm 0.00 \mu\text{m}$ and $85.2 \pm 1.12 \mu\text{m}$). The result of the ANOVA indicates a significant difference between these two treatments (Table 2). Averaged over time, the mean percent difference in arm length relative to body length between low- and high-fed larvae was 11.88% for *S. franciscanus* and 4.53% for *S. purpuratus* larvae. These results do not support the evolutionary argument of the second hypothesis that larvae from a species that has evolved smaller eggs express a greater degree of plasticity than larvae from a species that has evolved larger eggs.

Interestingly, experimentally reducing the amount of energy available to *S. franciscanus* larvae via blastomere separation did not result in the production of larvae that expressed a degree of plasticity comparable to *S. purpuratus* larvae. Half-size *S. franciscanus* larvae developed from individual blastomeres that were smaller than full-size *S. purpuratus* eggs ($<70 \mu\text{m}$ vs $81.8\text{--}85.2 \mu\text{m}$). Although half-size *S. franciscanus* larvae developed from comparable, yet slightly smaller eggs than full-size *S. purpuratus* larvae, they expressed a greater mean percent difference in relative arm length between low- and high-fed larvae when averaged over time (8.40% versus 4.53%, respectively).

Furthermore, halving the amount of energy available to a developing larva did not alter the pattern of plastic

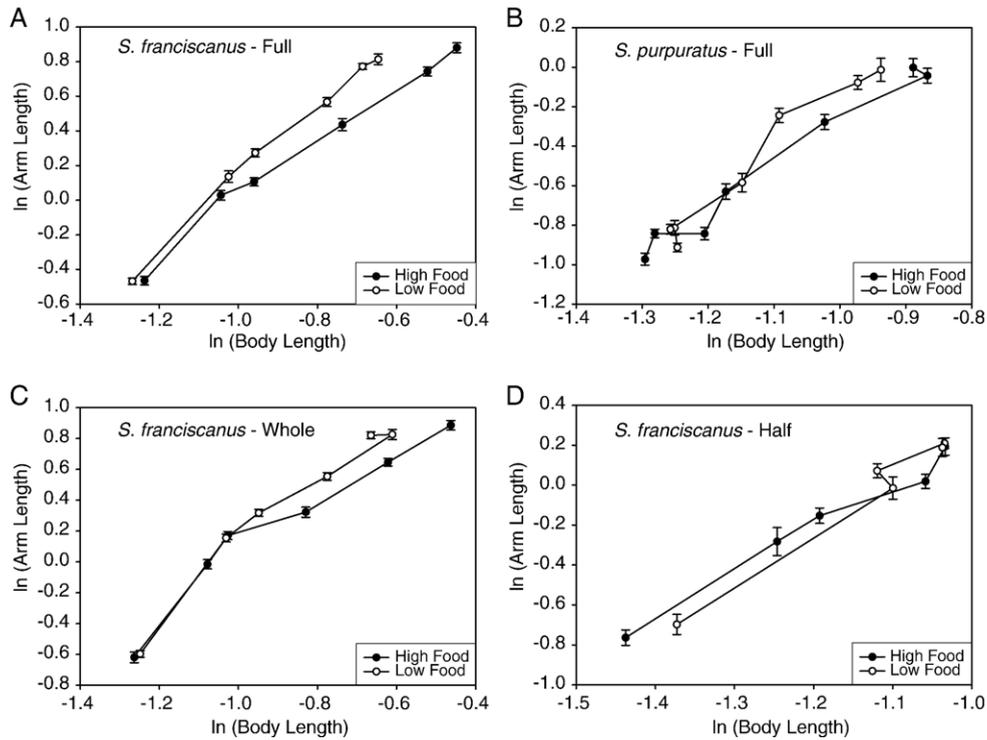


Fig. 3. Mean summed length of postoral and anterolateral arms (\pm 1SE) for high-fed (filled symbols) and low-fed (open symbols) larvae versus mean summed body length at midline (\pm 1SE). Arm and body length values for individual larvae were natural log-transformed before means were calculated. A) *Strongylocentrotus franciscanus* larvae from full-size eggs. B) *S. purpuratus* larvae from full-size eggs. C) *S. franciscanus* larvae from whole-size eggs: individual blastomeres did not dissociate during the blastomere separation treatment. D) *S. franciscanus* larvae from half-size eggs: individual blastomeres dissociated into half-size “eggs” during the blastomere separation treatment.

expression over time. Certainly there was variation in the degree of plasticity that was expressed by the different species and treatments over time (Fig. 4), but *S. franciscanus* larvae from the full-, whole-, and half-size treatments all exhibited a similar pattern as shown by the comparable trajectory of the degree of plasticity curves in Fig. 4. Although it is not surprising for full- and whole-size larvae to develop along a similar trajectory, it is interesting that half-size larvae followed a similar pattern and expressed degrees of plasticity comparable to larvae developing from ‘normal’ (full or whole) size eggs of the same species. Full-size *S. purpuratus* larvae, which develop from eggs that are comparable in size to half-size *S. franciscanus* larvae, exhibit a different pattern in degree of plasticity over time. These differences in response of arm length to food are captured in the results of the two ANOVAs: there was a significant species with food interaction term ($p=0.0012$) in the comparison between full-size *S. franciscanus* and *S. purpuratus* larvae, and a non-significant treatment with food interaction term ($p=0.6418$) in the comparison among *S. franciscanus* larvae from the three treatments.

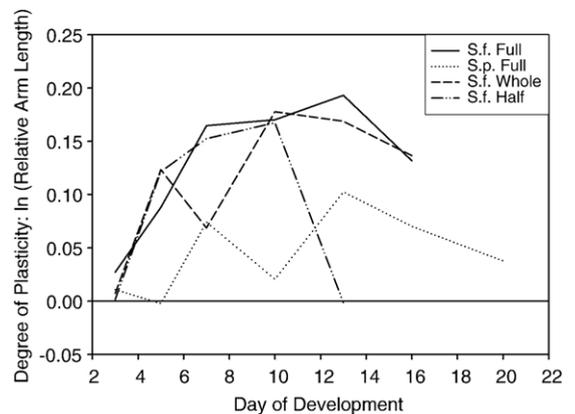


Fig. 4. Degree of plasticity of relative arm length for full-size *Strongylocentrotus purpuratus* and full-, whole-, and half-size *S. franciscanus* larvae over time (dotted, solid, dashed, dash-dotted lines, respectively). Degree of plasticity was calculated by subtracting the mean natural log-transformed arm:body length ratios expressed by larvae reared in the high food environment from the mean natural log-transformed arm:body length ratios expressed by larvae reared in the low food environment. Positive deviations from zero indicate low-fed larvae have longer arms, relative to body length, than high-fed larvae.

The similarity in developmental pattern among full-, whole-, and half-size *S. franciscanus* larvae, and their collective difference from the pattern expressed by *S. purpuratus* larvae, suggest that the evolutionary history and/or genetic predisposition of a species is, at least in this instance, more important than endogenous resource availability to the expression of plasticity. The trajectories displayed in Fig. 4 suggest that half-size *S. franciscanus* larvae may be genetically programmed to express a pattern of plasticity unique to *S. franciscanus*. Half-size larvae are able to maintain a level of plasticity that is comparable to normal-size larvae through day 10. At this time, lack of endogenous resources may limit the degree of plasticity (of arm length relative to body length) that can be expressed by low-fed, low endogenous energy, half-size larvae. Alternatively, *S. purpuratus* larvae, which develop from eggs that are comparable to half-size *S. franciscanus* 'eggs', exhibit minimal difference in relative arm length between low- and high-fed larvae over time, suggesting evolved differences between the two species in degree of plasticity.

Qualitative observation of Figs. 2 and 3 reinforces the notion that there is an interplay between the energy/materials and evolutionary history for the effect of egg size on the expression of plasticity in *Strongylocentrotus*. In Figs. 2 and 3, *S. franciscanus* larvae from all treatments exhibit a dramatic increase in arm length between days 3 and 5. The rapid increase is likely fueled by the large endogenous resources contained in the egg of this species because this pattern is apparent in both low- and high-fed larvae. Although large endogenous resources may fuel this pattern, it is clearly a pattern evolved by *S. franciscanus* because half-size *S. franciscanus* larvae exhibit the same pattern. Half-size *S. franciscanus* larvae do not display a pattern similar to *S. purpuratus*, which are comparable in egg size. *S. purpuratus* larvae display a more gradual increase in arm length during early development, reflecting the smaller amount of egg-bound energy, and an evolved pattern of plasticity that is different than the pattern exhibited by *S. franciscanus*.

Full-size *S. franciscanus* and *S. purpuratus* larvae exhibit clear differences in the pattern of plastic expression of relative arm length over time. However, adults from these two species were collected from the same location and their larvae co-occur in the same planktonic habitat; most environmental characteristics, e.g. food availability, levels of predation, etc. may be expected to exert similar selective pressures on larvae from either species. What can explain the difference in the patterns of plasticity adopted by each species?

To minimize mortality, larvae are presumed to be under strong selection to decrease development time spent in the plankton by increasing food assimilation (Rumrill, 1990;

Lamare and Barker, 1999). If this is indeed the case, then strategies that increase food capture under low food conditions, e.g. expressing a high degree of arm length plasticity, would support this hypothesis. However, life in the plankton may not be as dangerous as previously thought, as rates of larval predation may be lower than recognized (Allen and McAlister, 2007). Using tethered crab megalopae and flavored agarose pellets as baits, these authors found bait loss rates (due to predation) that were 12–25 times greater on the benthos than the plankton. If this result is representative, then selection to increase number of progeny, consequently decreasing the amount of endogenous materials provided to a given egg, may be stronger than selection to decrease development time in the plankton. The results of my study suggest that this may be the strategy adopted by *S. purpuratus*.

Emlet et al. (1987) reviewed size at settlement data for echinoid species with planktotrophic larvae and found that over a wide range of egg sizes, size at settlement was relatively constant. Furthermore, Doughty (2002) found that plasticity and maternal provisioning strategies can coevolve to help larvae cope with unpredictable larval environments. There may be multiple viable strategies to increase food consumption to attain settlement competency, if the rates of planktonic predation are relatively low. Evolving a larger egg size, trading-off progeny number, and increasing food assimilation by expressing a higher degree of plasticity, which results in a decrease of development time may be one strategy, as exhibited by *S. franciscanus*. Alternatively, evolving a smaller egg size, increasing progeny number, and expressing a lower degree of plasticity, which results in an increase in development time may be another, as exhibited by *S. purpuratus*.

4.1. Egg size, plasticity, and life-history evolution

The results of the present study differ with those of Podolsky and McAlister (2005) and Reitzel and Heyland (2007). Podolsky and McAlister's (2005) study of ophiuroid pluteus larvae indicated that the two smaller-egged species exhibited plasticity of larval arm length and the two larger-egged species did not. Reitzel and Heyland (2007) also found that larvae of the two smaller-egged species (*Mellita tenuis* and *Clypeaster subdepressus*) exhibited a significantly higher plastic response to low food conditions than the larger-egged species (*Leodia sexiesperforata*).

Initial egg size may account for the differing results among the present study and those of Podolsky and McAlister (2005) and Reitzel and Heyland (2007). Mean egg diameters of the four species of ophiuroids in the genus *Macrophiothrix* used by Podolsky and

McAlister (2005) were 147, 155, 166, and 230 μm . In Reitzel and Heyland's (2007) study, mean egg diameters were as follows: *M. tenuis* 99, *C. subdrepessus* 150, and *L. sexiesperforata* 191 μm . Mean egg diameters of the two *S. franciscanus* (122.7 and 124.7) and two *S. purpuratus* (81.8 and 85.2) individuals used in the present study are most comparable in size to *M. tenuis* and smaller than all of the other species in the two studies. Only the present study quantifies the degree or level of plasticity expressed by species with relatively small egg sizes.

Reported mean egg diameters of other species in which plasticity has been demonstrated are generally less than approximately 170 μm (Strathmann et al. 1992; Hart and Strathmann, 1994; Eckert, 1995; Bertram and Strathmann, 1999). Reitzel and Heyland (2007) suggest that planktotrophic species with a very high degree of maternal provisioning (*L. sexiesperforata* in their study; *Encope michelini*, Eckert, 1995) have decreased plastic expression. However, among planktotrophic species with 'smaller' egg diameters that fall within the range presented above, increased maternal provisioning may confer increased capacity for plastic expression. Furthermore, the degree and pattern of plastic expression may well be closely associated with the developmental strategy (within planktotrophy) of a given species (as discussed above), the amount of endogenous energetic reserves (egg size), and selection from environmental variables that are unique for a population from a given location. Other life-history parameters such as longevity, maximum adult size, age at 1st reproduction, etc. must also be taken into account.

Strathmann et al. (1992) proposed that plasticity of exogenous feeding structures may be associated with the evolution of large egg size, the loss of feeding structures, and the adoption of non-feeding development in some species. Bertram and Strathmann (1998) investigated whether endo- and exogenous food resources provide the same stimuli to developing *Strongylocentrotus droebachiensis* larvae. They found that larvae developing from the smaller eggs (153 μm) of food-limited mothers did not produce larger feeding structures than larvae developing from the larger eggs (159 μm) of food-satiated mothers. The authors suggested that changes in egg size alone may not lead to the loss of feeding structures, but that preexisting developmental plasticity may provide a mechanism for a coordinated suite of morphogenetic changes that leads to the evolution of non-feeding. In addition, unpublished work by Strathmann and Bertram (R.R. Strathmann, pers. comm.) in which egg volume of *S. purpuratus* was doubled by egg fusion to compare development with *S. droebachiensis* revealed that interactions between egg size and food supply did not over-

ride inter-specific differences in development of larval and juvenile structures. Their results suggested that an evolutionary increase in egg size alone does not result in the acceleration of the formation of juvenile structures and that other genetic changes are responsible for the evolution of non-feeding. In light of the differences in the patterns of plasticity exhibited by *S. franciscanus* and *S. purpuratus* in the present study, manipulations of egg size alone are clearly not sufficient to supercede genetic differences among species. Exogenous treatment with hormones (e.g. thyroxine), which has been shown to accelerate larval development (Heyland and Hodin, 2004; Heyland et al. 2004), coupled with egg size manipulations and/or genetic modification, may help to elucidate the mechanisms responsible for an evolutionary transition from feeding to non-feeding.

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