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Reduction in morphological plasticity in echinoid larvae: relationship of plasticity with maternal investment and food availability

Adam M. Reitzel[1]* and Andreas Heyland[1,2]

[1]*Department of Zoology, University of Florida, Gainesville, FL 32611 and* [2]*The Whitney Laboratory for Marine Biosciences, University of Florida, Saint Augustine, FL 32080, USA*

ABSTRACT

Hypotheses: If phenotypic plasticity is costly or not beneficial to marine invertebrate larvae, then increased maternal investment into eggs should result in decreased plastic responses. If genetic assimilation is a mechanism for the evolutionary transition from planktotrophic to lecithotrophic larvae, then species with larger eggs will have reduced overall plasticity and growth trajectories similar to highly fed larvae from a species that exhibits plasticity.

Organisms: Larvae reared from three subtropical echinoid species, *Mellita tenuis*, *Clypeaster subdepressus*, and *Leodia sexiesperforata*, collected from the Gulf coast of Florida that differ in degree of maternal investment into eggs.

Methods: We reared larvae from each species at three food concentrations in laboratory cultures. We measured two larval structures (post-oral arm length and stomach size) on three occasions (1, 3, and 5 days after fertilization) and statistically compared these characters to determine: (1) within-species plastic responses to food environment and (2) between-species plastic responses to identical feeding treatments.

Results: Larvae of *M. tenuis* and *C. subdepressus*, the two species with smaller eggs, both expressed plasticity of larval arms (elongated arms under low food conditions) and stomachs (smaller stomachs under low food conditions) early in development, whereas *L. sexiesperforata* larvae only showed significant changes in stomach size on the last day of measurement in the highest food treatment. Comparisons among species showed that larvae developing from smaller eggs had a significantly higher plastic response to exogenous food than larvae developing from large eggs.

Keywords: larva, maternal investment, phenotypic plasticity, pluteus.

INTRODUCTION

Phenotypic plasticity is the environmentally induced expression of different phenotypes by a single genotype (Schlichting and Pigliucci, 1998; West-Eberhard, 2003). Plasticity is expected to evolve

* Address all correspondence to Adam Reitzel, Department of Biology, Boston University, 5 Cummington Street, Boston, MA 02215, USA. e-mail: reitzel@bu.edu

when the organism is likely to encounter a heterogeneous environment, the environment can be interpreted to produce a matching phenotype, and the phenotypic responses result in a higher fitness compared with an organism that does not express the plastic phenotype (DeWitt *et al.*, 1998). The evolution of plasticity is constrained by both costs and limits of plasticity (DeWitt *et al.*, 1998) that result in a reduction in the fitness of a genotype as a consequence of expressing a phenotype in a particular environment through a plastic rather than fixed developmental process (Van Tienderen, 1997; DeWitt *et al.*, 1998). Costs and limits to plasticity have been detected in empirical studies [e.g. costs (Relyea, 2002; Fischer *et al.*, 2004; Huber *et al.*, 2004; Relyea and Auld, 2004) and limits (Padilla and Adolph, 1996; Langerhans and DeWitt, 2002)]. We can infer historical impacts of costs and limits of plasticity on life histories through use of the comparative method to test for plastic responses to environmental conditions in related organisms that differ in specific life-history characters.

One area of phenotypic plasticity research is how animals modulate features of their ingestive and digestive system in response to diet quantity and/or quality (e.g. Mayzaud, 1986; Karasov and Cork, 1996; Pfenning and Murphy, 2002). Marine invertebrate larvae express morphological phenotypic plasticity in response to their nutritional environment (e.g. Boidron-Metairon, 1988; Strathmann *et al.*, 1993). Specifically, phenotypic plasticity in sea urchin and sand dollar larvae (Phylum Echinodermata; Class Echinoidea) has been widely investigated providing a comparative framework for studying the evolution and maintenance of phenotypic plasticity of ingestive structures and digestive systems (e.g. Fenaux *et al.*, 1994; Hart and Strathmann, 1994; Miner, 2005). A plastic phenotype in either ingestive or digestive structures that increases nutrient acquisition efficiency would decrease pelagic development time, thereby decreasing the probability of larval mortality (Rumrill, 1990; Lamare and Barker, 1999). This selection pressure alone could favour the evolution of morphological plasticity because the planktonic environment is food limited and heterogeneous (Conover, 1968; Olson and Olson, 1989; Harms *et al.*, 1991).

To date, studies of phenotypic plasticity for echinoid larvae have largely investigated species whose larvae develop from poorly provisioned eggs. Echinoid larvae develop from a continuous range of maternal investments (Emlet *et al.*, 1987; McEdward, 2000) but metamorphose at a consistent size (Emlet *et al.*, 1987; Levitan, 2000), indicating different exogenous energy requirements. Larvae from less provisioned eggs have to collect a relatively larger amount of food from the environment than more richly provisioned larvae. Thus, larvae developing from the latter would be less likely to express phenotypic plasticity as they are less dependent on exogenous food to reach metamorphic competence, especially if the costs of plasticity are high (Relyea, 2002). Conversely, larvae developing from poorly provisioned eggs require more resources during the larval stage and spend longer periods in a heterogeneous feeding environment thereby favouring plasticity. These two predictions provide the opportunity to determine experimentally if maternal investment in offspring correlates with differential expression of larval plasticity in variable feeding environments.

To better understand how morphological plasticity varies between species and between morphological characters within a species, we tested two hypotheses concerning the expression of morphological plasticity in echinoid larvae: (1) Do echinoid larvae from three species differing in maternal investment respond to three food environments in similar ways? (2) For a species that exhibits morphological plasticity, are both ingestive (i.e. larval arm length) and digestive structures (i.e. stomach size) plastic in complementary ways for nutrient acquisition?

MATERIALS AND METHODS

Collection and culturing larvae

We used three related, subtropical echinoids that differ in maternal investment but share common ecological habitats in coastal Florida. Although these three species are all irregular echinoids in the Order Clypeasteroida, *Mellita tenuis* and *Leodia sexiesperforata* are more closely related to one another (Suborder Scutellina, Family Mellitidae) with *Clypeaster subdepressus* as an outgroup [Suborder Clypeasterina, Family Clypeasteridae (Littlewood and Smith, 1995)]. Adult *M. tenuis* and *C. subdepressus* were collected using SCUBA west of Cedar Key, Florida in May 2001. Adult *L. sexiesperforata* were collected by snorkeling off Long Key, Florida in June 2001. Adult animals were maintained in recirculating seawater aquaria for less than a week before the experiment.

Spawning was induced using an intra-coelomic injection of 0.55 M KCl. We present a brief description of the embryo and larval rearing process below; further details of the procedures followed can be obtained from Strathmann (1987). For each species, eggs from a single female were collected and washed three times. Sperm from a single male was collected 'dry' and then diluted for fertilization. Eggs were fertilized (>95%) and incubated at 28°C in 2-litre glass beakers. Egg diameters were measured post-fertilization with an ocular micrometer measuring to the nearest micrometer. Larval cultures for all three species were maintained at concentrations of one larva per 4 ml of 0.45-μm Millipore filtered natural seawater. Water in each larval culture was changed every 2 days. Larvae were fed the phytoplanktonic green alga *Dunaliella tertiolecta* (Butcher), cultured in f/2-enriched seawater medium (Guillard, 1975).

Egg biochemistry

The mean protein, carbohydrate, and lipid contents ($\mu g \cdot egg^{-1}$) of fertilized eggs were determined with colorimetric techniques (for a detailed description, see Reitzel *et al.*, 2005) to verify that egg size correlated with egg energy content. Previous studies have shown that egg size is not necessarily an accurate indicator of maternal investment (McEdward and Carson, 1987; McEdward and Morgan, 2001), although among species, larger eggs typically have a greater degree of energy (Jaeckle, 1995; McEdward and Morgan, 2001; Pernet and Jaeckle, 2004). Five replicate samples of fertilized eggs were prepared in micro-grinders (100–1000 μl capacity, Fisher Scientific). Excess seawater was removed from the eggs with a micropipette. Eggs were washed once with distilled water and then homogenized with a glass pestle. Proteins were quantified with the Coomassie brilliant blue G-250 binding assay (Bradford, 1976). Carbohydrates were assayed with the phenol-sulphuric acid method (Dubois *et al.*, 1956). Lipids were extracted with chloroform and methanol (Christie, 1982) and assayed using a modification of the acid-dichromate oxidation technique (Parsons *et al.*, 1984).

Morphological plasticity

To assess morphological plasticity in larvae of these three echinoid species, larvae were fed one of three food treatments: low food (2 cells $\cdot \mu l^{-1}$), medium food (6 cells $\cdot \mu l^{-1}$), and high food (8 cells $\cdot \mu l^{-1}$), with three replicate beakers per treatment. At 1, 3, and 5 days post-fertilization, approximately 20 larvae were sampled per culture and fixed with 1 ml of 4% formalin per 5 ml of seawater. Ten of these larvae per replicate were randomly sampled

and measured for three characters – post-oral arm length, and stomach length and width – that were used to calculate stomach cross-sectional area. Measurements were based on straight-line distances in three-dimensional space by using a compound microscope integrated with a digitizing tablet (McEdward, 1985). The skeletal and stomach dimensions were measured on larvae that were dehydrated in ethanol, then cleared by immersion in clove oil. This procedure renders the larval tissues transparent, allowing accurate measurement of both the stomach and skeleton. We performed a series of measurements of various development stages at each step of the sample preparations that showed the protocol had no effect on larval features. For *C. subdepressus* and *M. tenuis*, 10 individual larvae were measured from each replicate of each treatment. For experiments with *L. sexiesperforata*, fewer embryos were obtained; therefore, 10 individuals were pooled from the three replicate beakers and measured from each treatment.

Statistical analysis

We tested for differences in egg size and biochemical composition of eggs using analysis of variance (ANOVA) followed by Bonferroni *post-hoc* comparisons. Similarly, we used ANOVA to determine significant differences in post-oral arm length and stomach cross-sectional area at each measured time point for each species. Significant differences between feeding treatments for a species at the same time post-fertilization would indicate expression of phenotypic plasticity. We compared within-species growth trajectories for the two extreme feeding treatments (2 cells $\cdot \mu l^{-1}$, 8 cells $\cdot \mu l^{-1}$) with regression estimation (SYSLIN in SAS).

We tested the effect of feeding concentrations and species on phenotypic plasticity using ANOVA commands in SPSS. We applied a full factorial model to the data set using species, food concentration (treatment), age (time after fertilization), and replicate beakers as fixed factors, stomach size as a covariate, and post-oral arm length as a dependent variable. We analysed each age group (day 1, 3, 5) separately. In the full factorial model, the interaction term species × treatment reflects how species respond to feeding treatments.

RESULTS

Egg diameter and biochemical quantification of lipids, carbohydrates, and proteins showed significant differences for the three echinoid species (Table 1). *Leodia sexiesperforata* had significantly larger eggs with higher egg energy than either *C. subdepressus* or *M. tenuis*. *Clypeaster subdepressus* had intermediate egg size and egg energy, while *M. tenuis* had the most poorly provisioned eggs, corresponding to its smallest size.

Figure 1 shows reaction norms for two morphological characters (post-oral arm length and stomach area) of the three species reared at three different food concentrations for three different larval ages. Statistical comparisons of mean arm length and stomach cross-sectional area between food concentrations at each sampled date showed similar responses for *M. tenuis* and *C. subdepressus* but not for *L. sexiesperforata*.

At 1 day post-fertilization, when larvae had been feeding for less than 6 h, stomach area differed significantly in the two lower food treatments when compared with the highest for both *M. tenuis* and *C. subdepressus*. Post-oral arm length did not differ significantly in any of the species on day 1. Neither stomach size nor post-oral arm length differed in any of the three treatments for *L. sexiesperforata*.

Table 1. Egg diameter and energy comparison among *Mellita tenuis*, *Clypeaster subdepressus*, and *Leodia sexiesperforata*

	Mellita tenuis	*Clypeaster subdepressus*	*Leodia sexiesperforata*	ANOVA
Egg diameter	99.14 ± 2.09^a	150.26 ± 4.04^b	191.08 ± 5.42^c	$F_{2,57} = 2542.93$, $P < 0.0001$
Lipids	0.161 ± 0.0622^a	0.259 ± 0.026^b	0.446 ± 0.069^c	$F_{2,12} = 37.41$, $P < 0.001$
Carbohydrates	0.0518 ± 0.0368^a	0.0775 ± 0.0376^a	0.106 ± 0.0122^b	$F_{2,12} = 17.92$, $P < 0.0001$
Proteins	0.0432 ± 0.00376^a	0.0889 ± 0.0150^b	0.284 ± 0.012^c	$F_{2,12} = 223.88$, $P < 0.001$

Note: Values represent mean egg biomass in micrograms for all three species for the three energy types measured ±1 standard deviation. Significance was assessed at $P < 0.05$. Different superscript letters indicate significant differences in egg size or biochemical composition between species.

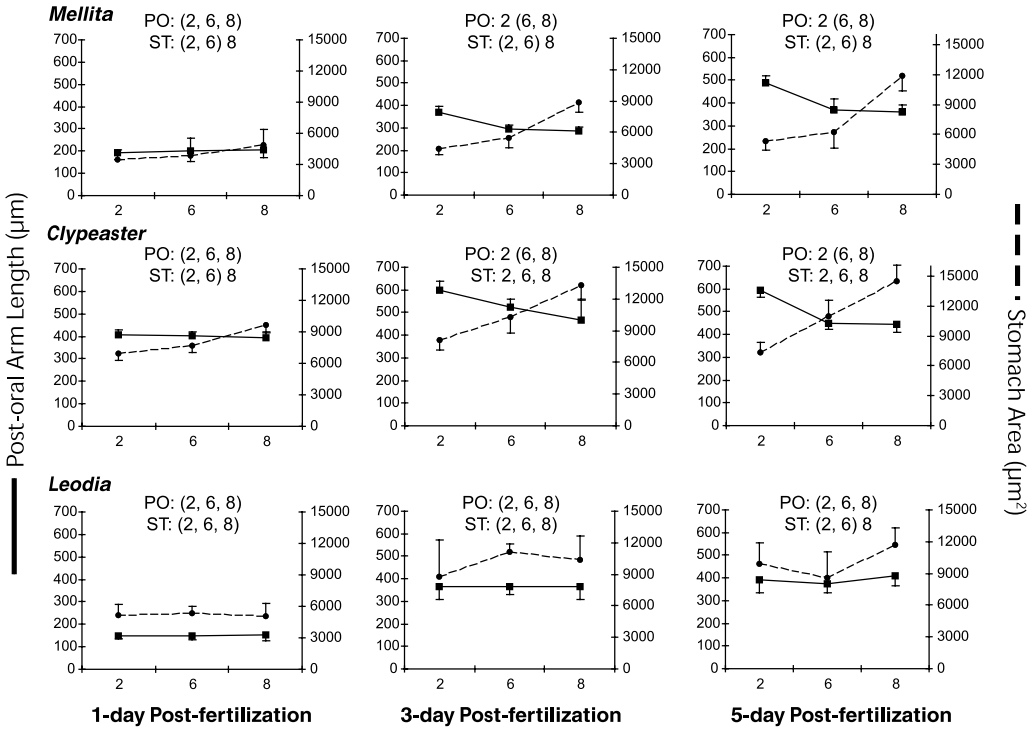

Fig. 1. Reaction norms for two larval characters (post-oral arm length, stomach cross-sectional area) in *M. tenuis*, *C. subdepressus* and *L. sexiesperforata* larvae measured 1, 3, and 5 days post-fertilization. We indicate differences between feeding treatments (PO = post-oral arm length, ST = stomach area), as indicated by Bonferroni *post-hoc* comparisons, by grouping treatments that did not differ significantly ($P < 0.05$) in parentheses and those that did are separated by commas without parentheses. Values are mean ± standard deviation.

At 3 days post-fertilization, *M. tenuis* and *C. subdepressus* larvae expressed plasticity in both stomach size and post-oral arm length in the expected inverse relationship (Miner, 2005). For stomach area, the highest food concentration resulted in significantly larger stomachs compared with the other two treatments. We observed significant differences between all feeding treatments in *C. subdepressus*, whereas for *M. tenuis* the two lower feeding treatments had statistically indistinguishable stomach sizes. Post-oral arm length was also significantly different between feeding treatments for *M. tenuis* and *C. subdepressus* but, in contrast to stomach area, larvae reared in the two higher food treatments were statistically indistinguishable for both species. Less food resulted in significantly longer arms when compared with the two higher food treatments. *Leodia sexiesperforata* did not express plasticity in either structure 3 days post-fertilization.

Mellita tenuis and *C. subdepressus* larvae maintained significant plasticity at 5 days post-fertilization in the same relationship as measured at 3 days post-fertilization. *Leodia sexiesperforata* again did not show plasticity in post-oral arm length between feeding treatments, but we did detect a plastic response in stomach size with larvae reared in the highest food concentration treatment having larger stomachs than those in the two lower food treatments.

We plotted growth trajectories for each species to compare post-oral arm length and stomach cross-sectional area (Fig. 2). *Mellita tenuis* and *C. subdepressus* larvae both diverged in low and high food treatments. For *M. tenuis* and *C. subdepressus*, individuals in the low food treatment (2 cells $\cdot \mu l^{-1}$) increased in post-oral arm length, with little growth in stomach area. Conversely, in the high food treatment (8 cells $\cdot \mu l^{-1}$), larvae grew larger stomachs, with less extension of the post-oral arms. The slopes of growth trajectories between these food treatments were significantly different for both species (*M. tenuis:* $F_{1,176} = 6.96$, $P = 0.0091$; *C. subdepressus*: $F_{1,176} = 7.62$, $P = 0.0064$). On the other hand, growth of *L. sexiesperforata* was similar in each food treatment and growth trajectories were statistically indistinguishable ($F_{1,56} = 0.01$, $P = 0.9280$).

In the full factorial ANOVA, the interaction term of species × treatment was statistically significant for all three larval ages (Table 2), indicating that the plastic response to food treatment is different for the three species studied. Additionally, we tested the interaction between post-oral arm length and stomach size. The species × food treatment × stomach size (covariate interaction) was significant for all three times investigated (day 1: $F_{9,209} = 298.42$, $P < 0.001$; day 3: $F_{9,209} = 294.12$, $P < 0.001$; day 5: $F_{9,209} = 113.04$, $P < 0.001$).

DISCUSSION

The phenotypic plasticity in post-oral arm length and stomach size shows that ingestion and digestion are flexible for nutrient acquisition and digestion in a heterogeneous environment. Structural responses that increase ingestion rate (i.e. morphological plasticity in arm length) and digestion or storage have both received considerable attention. Based on nutrition acquisition research in other taxa (e.g. Penry and Jumars, 1987; Martinez del Rio *et al.*, 1994; McWilliams and Karasov, 2001), we would expect morphological differences in either ingestive or digestive structures of larvae reared in different food environments depending on what stage limits nutritional uptake. Miner (2005) showed a trade-off between arm length and stomach size in two echinoid species of the genus *Strongylocentrotus*. Heyland and Hodin (2004) and Strathmann *et al.* (1992) also provided evidence for a trade-off between larval characters and the juvenile rudiment in the sand dollar *Dendraster excentricus*. In the present study,

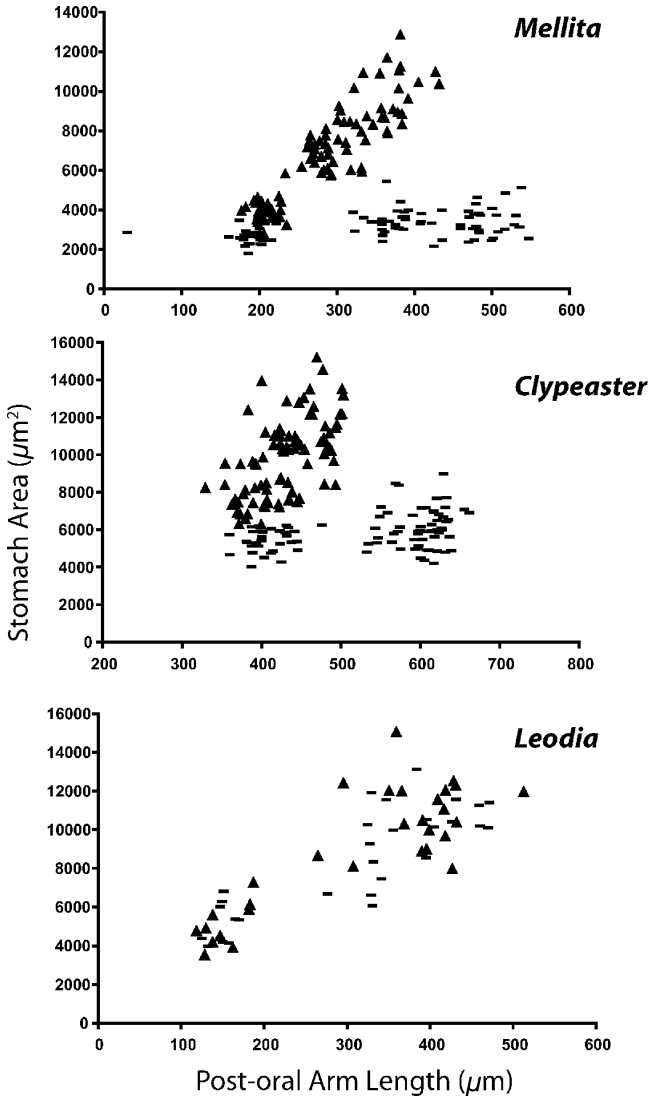

Fig. 2. Larval growth trajectories of *M. tenuis*, *C. subdepressus*, and *L. sexiesperforata* in two food concentrations over 5 days. Each data point is an individual's post-oral arm length and stomach area measurements. *Mellita tenuis* and *C. subdepressus* display significantly different growth trajectories as shown by the separation of each treatment, with one treatment displaying disproportionate growth in one character but not the other. Conversely, in *L. sexiesperforata*, both feeding treatments had similar growth trajectories.

we found a similar difference between arm length and stomach area with high and low food concentration for two species (*M. tenuis* and *C. subdepressus*), but not a third (*L. sexiesperforata*; Figs. 1, 2). Larvae of the two species that expressed plasticity showed similar convergent morphologies in the low and high food environments, while *L. sexiesperforata* developed the same morphology regardless of food environment.

Table 2. Analysis of the interactive effects of species, feeding treatment, and experimental replicate on larval post-oral arm length using full factorial ANOVA

		Day 1		Day 3		Day 5	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Species	2	184.2	0.000	207.6	0.000	99.68	0.000
Feeding treatment	2	0.788	0.456	89.54	0.000	81.79	0.000
Replicate	2	1.320	0.270	1.482	0.230	1.002	0.369
Species × feeding treatment	4	2.812	0.027	22.12	0.000	20.85	0.000
Species × replicate	2	0.231	0.794	0.186	0.830	0.435	0.648
Feeding treatment × replicate	4	8.102	0.000	0.826	0.510	0.714	0.583
Species × feeding treatment × replicate	4	4.778	0.001	2.537	0.042	1.058	0.379
Stomach area	1	0.716	0.398	3.831	0.052	6.772	0.010

Note: Results were analysed separately for the three developmental periods (1, 3, and 5 days post-fertilization).

Significant differences for the two larval characters did not directly represent a simple trade-off when comparing the three treatments. *Mellita tenuis* larvae in the intermediate food treatment at 3 and 5 days post-fertilization had arm lengths statistically indistinguishable to the high food treatment but stomach areas comparable with the low food treatment. A similar response was seen in *C. subdepressus* with the exception that the stomach size for the intermediate food was significantly different from and intermediate to both low and high food, although the size was quantitatively more similar to the low food treatment (Fig. 1). From a functional perspective, it would appear that *M. tenuis* and *C. subdepressus* larvae in the intermediate food concentration produce an intermediate plastic phenotype, short arms and small stomachs. If larvae are ingesting relatively less food, reduced stomach size would be a potentially adaptive response to reduced ingestion. Still, given that larvae are presumed to be under selection to maximize food assimilation to minimize development time and thus reduce larval mortality (Rumrill, 1990), larvae should produce phenotypes that maximize food uptake/assimilation, not minimize it. This result raises the question whether the intermediate phenotype is a mismatch to the environment or a functional solution when ingesting less food where a reduction in development time is not under strong selection.

Larval arm length and stomach size in the two extreme food concentrations from our study as well as a number of previous studies (e.g. Boidron-Metairon, 1988; Miner, 2005) showed an inverse relationship for feeding and digestive structures. Longer arms for increased ingestion are typically paired with smaller stomachs and vice versa (exception in intermediate food concentration, see above). There are no reported mechanisms for how this coordinated plastic response between morphological characters would occur or what type of signalling network might be involved. Is one part of the food assimilation (i.e. ingestive or digestive structures) leading the morphological change with the other following suit? Plastic responses in echinoid larvae first manifest in the stomach (Miner, 2005; this study), suggesting that a plastic response is at least initially expressed in these structures. The developmental coordination of larval characters may be facilitated by hormones that serve as signals of the larva's environment. Recent work with thyroxine in echinoid development suggests that this hormone could serve this function. Application of exogenous thyroxine

replicates the plastic response generated by food concentration (Heyland and Hodin, 2004; Heyland *et al.*, 2006). The potential role for thyroxine in coordination of the plastic response in echinoid larvae suggests a plausible mechanism that could be tested further.

Our data represent the second reported case of an echinoderm larva with a high degree of maternal investment that does not express morphological plasticity in response to different food concentrations [*Encope michelini* (Eckert, 1995; George, 1999)]. Increased maternal investment results in a decreased dependence on exogenous food and thus a possible release from selection for plasticity in larval morphology in response to food environment. It is generally assumed that plasticity carries costs. Otherwise, all organisms could be perfectly plastic in heterogeneous environments (DeWitt *et al.*, 1998). For echinoid larvae, the benefits of plasticity (increased water clearance rate for elongation of larval arms, presumed increased digestion efficiency or storage with enlargement of stomach) are either weighed against a number of costs or simply provide little or no benefit when expressed. Potential costs may include the cost of producing larval structures, costs associated with environment signal reception, and genetic costs associated with the expression of plasticity (DeWitt *et al.*, 1998). Alternatively, and because no costs have been determined experimentally for echinoid larvae, the lack of plasticity may also indicate that there is little or no benefit for expressing plasticity in *L. sexiesperforata* or other species with a high degree of maternal investment.

Larvae developing from large eggs require little exogenous food to reach metamorphic competence. Emlet *et al.* (1987) reviewed size at settlement in echinoid species with planktotrophic larvae and revealed that juvenile size at settlement is relatively constant over a wide range of egg sizes. From this analysis, the primary difference between species developing from a range of maternal investments is a reduction of development time with increasing maternal investment. Consistent with this trend, data from our laboratory indicate that: (1) larvae from *L. sexiesperforata* need only moderate food concentrations for less than 2 days to attain sufficient energy to metamorphose (A.M. Reitzel *et al.*, unpublished data); (2) that feeding at different concentrations of food has little impact on time to metamorphosis or juvenile energy compared with more poorly provisioned species (Reitzel *et al.*, 2005); and (3) in experimental treatments with exogenous thyroxine, larvae have sufficient energy from the egg to metamorphose without any exogenous food (Heyland *et al.*, 2004). In addition, a small proportion of larvae from a single clutch will metamorphose without thyroxine or food (Heyland *et al.*, 2004), similar to work with *Encope michelini* (Eckert, 1995). The application of thyroxine to *L. sexiesperforata* larvae also suggested that these larvae have retained the potential for a shift in development of larval and juvenile structures. The difference in developmental trajectories between larvae fed algae where little or no plasticity is detected versus larvae exposed to exogenous hormones where plasticity is detected could indicate that capacity for a plastic response may be retained and that only upstream steps in signalling have been lost. Our results support the hypothesis that the lack of any significant benefit or potentially undetermined costs incurred from producing variable larval morphology in a heterogeneous food environment may negate any benefits to an organism with such weak dependence on exogenous food.

Evolution of non-feeding development through plasticity?

Plasticity in echinoid larvae has been suggested as a mechanism for the evolution of non-feeding, lecithotrophic larvae (Strathmann *et al.*, 1992). With selection favouring non-feeding

over feeding development, energy allocation could be shifted from larval to juvenile structures. The developmental trajectory for the juvenile structures may then undergo genetic assimilation to become expressed constitutively yielding non-feeding larvae.

Bertram and Strathmann (1998) did not support this hypothesis based on intraspecific comparisons of larval development from eggs of different investment in the echinoid *Strongylocentrotus droebachiensis*. In this study, Bertram and Strathmann (1998) suggested that plasticity could instead provide a mechanism for coordinated changes in morphogenesis in the evolution of non-feeding larval development. Although we did not measure any juvenile structures *per se* in this study, our results in combination with our earlier work with thyroxine treatments (Heyland *et al.*, 2004) support this later hypothesis. Larvae developing from smaller eggs (e.g. *Mellita tenuis*) show a much higher degree of plasticity in larval-specific characters than larvae developing from large eggs [*L. sexiesperforata* (this study); *Encope michelini* (Eckert, 1995)]. We showed that *L. sexiesperforata* larvae expressed little or no plasticity in response to food concentration, but exogenous thyroxine treatment during development of this species does produce the shift in relative growth of larval and juvenile structures (Heyland *et al.*, 2004). Development of juvenile structures is heterochronically shifted in species with large maternal investments and with exposure to thyroxine – that is, juvenile structures develop earlier in the developmental trajectory (e.g. Eckert, 1995; Heyland and Hodin, 2004). A plastic response linking development of larval and juvenile stages coordinated by endogenous or exogenous hormones, potentially thyroxine, could result in allocation of resources from larval structures to the juvenile. Signalling and selection to accelerate juvenile development could become constitutive with the evolution of endogenous thyroxine synthesis (Saito *et al.*, 1998; Heyland *et al.*, 2004, 2006). Subsequent evolutionary changes to reduce and eventually remove genes or pathways involved in the development of larval-specific feeding structures could then result in non-feeding development.

CONCLUSION

Phenotypic plasticity is a trait subject to evolution and numerous studies have addressed how plasticity evolves, selection pressures associated with expression, and the underlying genetics (Scheiner and Lyman, 1991; Scheiner, 1993; Via *et al.*, 1995; DeWitt *et al.*, 1998; Van Buskirk and Schmidt, 2000; Fischer *et al.*, 2004; Huber *et al.*, 2004). Responses and degrees of plasticity can vary among populations (Schlichting, 1986) and may result in speciation (Pfenning and Murphy, 2002). The comparative method provides an insightful approach to identifying correlates for reduction or loss of plasticity in species or populations that vary in potentially significant life-history characters. Our results show that larvae developing from richly provisioned eggs have reduced phenotypic plasticity likely due to a decreased dependence on exogenous food. Larvae with a high maternal investment may have reduced morphological plasticity for nutrient assimilation as a consequence of reduced benefits or costs that are yet to be determined. Further work with echinoid larvae provides a fertile field for comparative studies of the evolution of phenotypic plasticity in planktotrophic larvae. Quantifying plasticity in a larger range of echinoids (and echinoderms) that develop from a range of maternal investments will provide a broader understanding of how offspring investment strategies influence larval development in variable food environments.

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