

# EVOLUTIONARY RESPONSES TO ENVIRONMENTAL HETEROGENEITY IN CENTRAL AMERICAN ECHINOID LARVAE: PLASTIC VERSUS CONSTANT PHENOTYPES

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**Do changes in food resources lead to evolutionary changes in phenotypic plasticity or in different constant phenotypes? I addressed this question by studying plasticity of larval feeding arms for “geminate species pairs” in three echinoid genera. These closely related species were geographically isolated when the Panamanian Isthmus raised 2.8–3.1 million years ago, creating two different food level environments: high but variable food levels in the eastern Pacific versus chronically low food levels in the western Caribbean. I reared larvae of geminate species in different replicated food environments for 10 days postfertilization, collected morphological measurements of individual arm and body lengths, and calculated degrees of plasticity of relative arm length for each species. In contrast to previous studies with temperate echinoids, there was no significant plasticity of arm length in either the Pacific or Caribbean species considered here. Caribbean species, however, had significantly longer relative arm lengths than Pacific species, regardless of food levels. These results suggest that historical changes in food levels have led to the evolution of constant rather than plastic differences between Pacific and Caribbean echinoids. The evolution of plasticity may be limited by the timing of reproduction or by egg size in this system.**

**KEY WORDS:** Constant phenotype, *Diadema*, echinoid, *Echinometra*, *Euclidaris*, evolution, geminate species pairs, larvae, phenotypic plasticity.

The expression of a phenotype is intricately associated with the environment in which an organism resides. In some cases, the phenotype expressed by a given genotype can be influenced by environmental conditions, a phenomenon known as phenotypic plasticity (Bradshaw 1965; Stearns 1989). Alternatively, a genotype can produce the same phenotype across environments, indicating that the expression of the phenotype is constant. In heterogeneous environments, phenotypic plasticity may allow an organism to maximize fitness (Gotthard and Nylin 1995); given appropriate genetic variability for plasticity and predictable environmental cues in a population, adaptive phenotypic plasticity is expected to evolve (Via et al. 1995). Conversely, expression of a constant phenotype is expected to confer high fitness and to evolve in en-

vironments with low heterogeneity and constant environmental characteristics.

The association between environmental changes and the expression of plasticity has been studied at several levels of evolutionary inquiry. Researchers have documented plasticity in response to different environments in many taxa (Boidron-Metairon 1988; Fenaux et al. 1988; Hart and Scheibling 1988; see reviews by Robinson and Wilson 1994; Skúlason and Smith 1995; Smith and Skúlason 1996; West-Eberhard 2003). In addition, studies have demonstrated variation in the degree of plasticity among populations (or species associations) in response to the degree of variation in the environment (DeBenedictis 1974; Kaitala 1991; Blouin 1992; Leips and Travis 1994; Buchholz and Hayes 2000;

Leips et al. 2000; Hayes 2002; Langerhans et al. 2003; Reinikainen and Repka 2003; Morey and Reznick 2004; Stauffer and Van Snik Gray 2004). However, few studies explore whether historical changes in environments are associated with the evolution of phenotypic plasticity or of different constant phenotypes (see Morey and Reznick 2004 for one example). What remains unknown in many systems are the relationships and times of divergence among different species, and how they have adapted to unique habitats since separation. These unknown variables make this level of evolutionary inquiry of greatest interest because no research has demonstrated an association between historical environmental changes and the repeated evolution of plastic or constant phenotypes. A comparison of phenotypic expression between close relatives that occupy habitats with different patterns of resource availability would therefore provide a crucial empirical test of the environmental factors underlying the evolution of alternative mechanisms for the expression of a phenotype.

A comparison of this type is provided by “geminate species pairs,” formed when previously continuous species were separated before or during the raising of the Panamanian Isthmus 2.8–3.1 million years ago (Keigwin 1982; Duque-Caro 1990). Geminate species pairs occur in multiple phyla (Jordan 1908), and although their time of divergence is variable (Knowlton et al. 1993; Knowlton and Weigt 1998; Marko and Jackson 2001), they have been evolving in isolation for at least 3 million years since the final rise of the Isthmus (Coates and Obando 1996). The rise of the Isthmus also separated the tropical western Atlantic (the western Caribbean Sea) and tropical eastern Pacific oceans, producing two environments that are markedly different with regard to productivity, which equates to food for plankton-feeding organisms. The eastern Pacific is characterized by strong, seasonal upwelling that produces variable yet predictably high phytoplankton food levels, whereas the western Caribbean experiences little upwelling, has low primary production, and is thus constantly nutrient poor and low in phytoplankton food (Glynn 1982; Keigwin 1982). Transisthmian geminate species offer a unique, replicated natural research system (Moran 2004) that can be used to address the evolution of adaptive phenotypic plasticity in response to the heterogeneity of food resource levels.

Morphological phenotypic plasticity in response to food level has been demonstrated in planktotrophic pluteus larvae from several species in the echinoderm class Echinoidea (Boidron-Metairon 1988; Strathmann et al. 1992; Hart and Strathmann 1994). These larvae depend on exogenous phytoplankton food, and, in response to low food availability, larvae increase the length of the ciliated band used for collecting food by growing longer larval arms; plasticity of ciliated band length is correlated with lengthening of skeletal arm rods in echinoplutei. Increased ciliated band length enhances larval ability to capture phytoplankton, and increases in ciliated band length under low food conditions

have been demonstrated to be adaptive because larvae with longer ciliated bands have greater maximum clearance rates (Hart and Strathmann 1994). In addition, by increasing ciliated band length, the larval surface-to-volume ratio increases, which could increase intake of dissolved organic matter (Manahan et al. 1983). For this reason, plasticity in arm length has been used as a measure of larval feeding history in the field (Strathmann et al. 1992). A recent study demonstrates genetic variation of larval arm length plasticity in response to food limitation in the echinoid *Lytechinus variegatus* (J. S. McAlister, unpubl. data).

Here I examine the evolution of phenotypic plasticity of larval feeding structures in response to differences in environmental heterogeneity for planktotrophic larvae of the echinoid geminate species pairs found off the coasts of Panama. For each geminate pair, one species lives in the highly productive but variable eastern Pacific, whereas the other inhabits the minimally productive and constant western Caribbean. Three sets of hypotheses can be made regarding the effect of food level heterogeneity on plasticity of larval arm length. First, the “Plasticity” Hypothesis posits that all species will exhibit some degree of phenotypic plasticity of larval arm length. This expectation can be justified by the fact that plasticity of larval arm length has been demonstrated in a large number of echinoid species in which it has been examined (Boidron-Metairon 1988; Hart and Scheibling, 1988; Strathmann et al. 1992; Hart and Strathmann 1994; Sewell et al. 2004; Reitzel and Heyland 2007).

Second, the “Differential Plasticity” Hypothesis posits that larvae evolving in the western Caribbean, which has constant low phytoplankton food levels, will exhibit low to no degrees of phenotypic plasticity of arm length. Conversely, larvae evolving in the eastern Pacific, characterized by variable phytoplankton food levels, will exhibit greater degrees of phenotypic plasticity of arm length. In support of this hypothesis, larval echinoid species from tropical or subtropical waters with low food levels show minimal plasticity (Boidron-Metairon 1988; Eckert 1995; Reitzel and Heyland 2007), whereas species from cold temperate waters with more variable food levels show greater degrees of plasticity (Boidron-Metairon 1988; Hart and Scheibling 1988). None of these studies are comparative or examined many taxa however.

Third, the “Constant Differences” Hypothesis posits that larvae evolving under constantly low food levels, characteristic of the western Caribbean, will grow longer arms relative to body length than larvae evolving in the variable food levels of the eastern Pacific. If phenotypic plasticity confers a benefit only in heterogeneous environments, then there may be no benefit of plasticity for larvae evolving in the homogeneous environment of the Caribbean. A better evolutionary strategy for resource acquisition may be to evolve longer arms under all conditions, especially if there is a cost of phenotypic plasticity (DeWitt et al. 1998). The number of examples in the literature is too small to thoroughly

test the patterns described by these hypotheses, nor have these ideas been tested in a rigorous phylogenetic context. My results indicate that historical changes in food availability can lead to the repeated evolution of differences in the expression of constant phenotypes between species, and suggest that the evolution of phenotypic plasticity may hinge in part on selection for other life-history characteristics associated with resource acquisition, for example, egg size.

## Materials and Methods

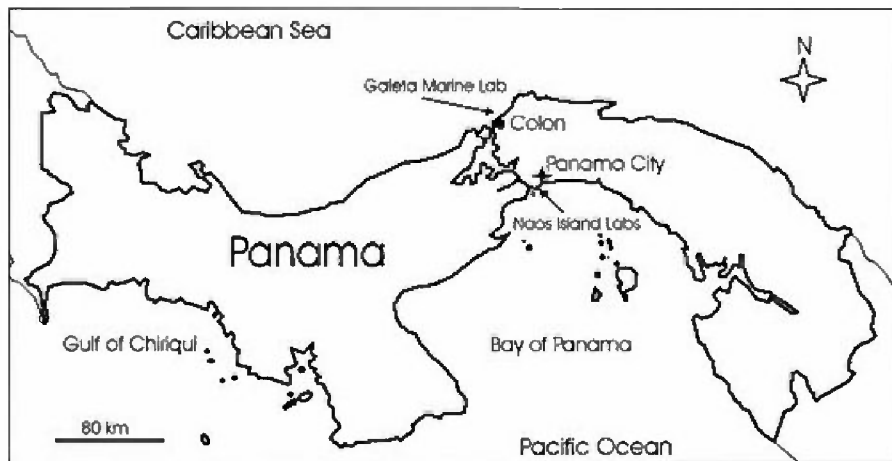
I investigated whether heterogeneity of food level is correlated with the expression of plastic and/or constant larval arm length by studying three geminate pairs of marine sea urchins in the genera *Diadema*, *Echinometra*, and *Eucidaris*. These species are found in coral reef habitats off the Caribbean and Pacific coasts of the Republic of Panama (Lessios 1979, 1981; Bermingham and Lessios 1993; McCartney et al. 2000). I performed two sets of experiments over the course of two summer field seasons in Panama. The first set of experiments examined larval morphological plasticity under two different food levels in two true geminate pairs, *Diadema antillarum* in the Caribbean with *D. mexicanum* in the Pacific and *Eucidaris tribuloides* in the Caribbean with *Eu. thouarsi* in the Pacific; genetic divergence among these species pairs is pegged to the final closure of the Central American Seaway approximately 2.8–3.1 million years ago (Lessios et al. 1999, 2001). In addition, I included in this experiment the *Echinometra* complex: *Ec. lucunter* and sister taxa *Ec. viridis* in the Caribbean with *Ec. vanbrunti* in the Pacific. The most recent common ancestor of *Ec. lucunter* and *Ec. viridis* is thought to be the geminate partner of *Ec. vanbrunti*, diverging approximately 3.1 million years ago; *Ec.*

*lucunter* and *Ec. viridis* diverged approximately 1.27–1.62 million years ago (McCartney et al. 2000). Although these three pairings are not the only echinoid geminates, they represent the genera with planktotrophic larvae that are most easily collected and spawned, and were therefore most amenable to this analysis. A second set of experiments examined the effects of food limitation on growth of *Ec. vanbrunti* and *Ec. viridis* larvae reared in one of five different food levels, including satiating and starvation conditions.

Adults of the sea urchins *D. mexicanum*, *Ec. vanbrunti*, and *Eu. thouarsi* were collected from the Pacific Ocean in June and July 2005 by SCUBA from populations located in waters off Isla Taboguilla near Panama City, Panama (see Fig. 1). Pacific species were placed in coolers filled with seawater and transported by boat to the Smithsonian Tropical Research Institute's (STRI) Naos Island Laboratories (Naos) near Panama City. Adults of their geminate species counterparts, *D. antillarum*, *Ec. lucunter*, *Ec. viridis*, and *Eu. tribuloides* were collected from the Caribbean Sea by snorkel in the vicinity of STRI's Galeta Marine Laboratory near Colon, Panama (see Fig. 1). Caribbean species were placed in disposable plastic containers (3–4 urchins per container) filled with a small amount of seawater. The containers holding Caribbean urchins were stacked in a cooler and transported by vehicle to Naos. All species were maintained in flow-through seawater aquaria at Naos; at this facility, effluent from aquaria containing Caribbean species is treated with bleach before discharge into Pacific coastal waters.

## LARVAL CULTURE

Gametes were obtained from adult urchins by injecting approximately 1 mL of 0.5M KCl through the peristomium into the body



**Figure 1.** Map of the Republic of Panama indicating the locations of the Smithsonian Tropical Research Institute's Galeta Marine Laboratory on the Caribbean coast and the Naos Island Laboratories on the Pacific coast (modified from map available at <http://www.enchantedlearning.com>). Adult urchins used to obtain gametes and produce larvae for this study were collected in waters in the immediate vicinity of Galeta Marine Laboratory and at Isla Taboguilla, located approximately 10 km offshore from Naos Island Laboratories.

**Table 1.** Initial mean ( $\pm 1SE$ ) egg diameters (**bold text; units = micrometers**) and volumes (**normal text; units = nanoliters**) for females of each species used to produce different larval families. Values were calculated using 25 eggs from each female.

Species	Ocean	Female used for each Full-sibling Family				Average
		1	2	3	4	
<i>D. antillarum</i>	C	<b>74.56 (0.45)</b> 0.22 (0.00)	N/A	N/A	N/A	<b>74.56 (0.45)</b> 0.22 (0.00)
<i>D. mexicanum</i>	P	<b>64.96 (0.34)</b> 0.14 (0.00)	<b>64.8 (0.45)</b> 0.14 (0.00)	<b>68.16 (0.19)</b> 0.17 (0.00)	N/A	<b>65.97 (0.27)</b> 0.15 (0.00)
<i>Ec. lucunter</i>	C	<b>83.2 (0.47)</b> 0.30 (0.00)	<b>82.08 (0.40)</b> 0.29 (0.00)	<b>79.84 (0.66)</b> 0.27 (0.00)	<b>87.28 (0.56)</b> 0.35 (0.00)	<b>83.1 (0.38)</b> 0.30 (0.00)
<i>Ec. Viridis</i>	C	<b>90.8 (0.41)</b> 0.39 (0.00)	<b>90.08 (0.38)</b> 0.38 (0.00)	<b>89.44 (0.45)</b> 0.38 (0.00)	N/A	<b>90.11 (0.25)</b> 0.38 (0.00)
<i>Ec. vanbrunti</i>	P	<b>67.84 (0.19)</b> 0.16 (0.00)	<b>67.76 (0.29)</b> 0.16 (0.00)	<b>69.76 (0.43)</b> 0.18 (0.00)	N/A	<b>68.46 (0.21)</b> 0.17 (0.00)
<i>Eu. tribuloides</i>	C	<b>92.64 (0.58)</b> 0.42 (0.00)	<b>92.16 (0.70)</b> 0.41 (0.00)	<b>93.44 (0.40)</b> 0.43 (0.00)	N/A	<b>92.74 (0.34)</b> 0.42 (0.00)
<i>Eu. thouarsi</i>	P	<b>86.08 (0.40)</b> 0.33 (0.00)	N/A	N/A	N/A	<b>86.08 (0.40)</b> 0.33 (0.00)

cavity. Eggs were collected and washed once in 0.45- $\mu\text{m}$  filtered seawater and sperm were collected by mouth pipette and kept on ice until use. Full-sibling larval families of all species were established by separately fertilizing eggs from one female with sperm from one male. Four separate full-sibling families were established for *Ec. lucunter*. Three separate full-sibling families were established for *D. mexicanum*, *Ec. vanbrunti*, *Ec. viridis*, and *Eu. tribuloides*. Due to the difficulty in finding reproductively mature adult females, one full-sibling family was established for both *D. antillarum* and *Eu. thouarsi*. Initial mean ( $\pm 1$  SE) egg diameters (means of 25 eggs each) and egg volumes (assuming a sphere) for females from each species are given in Table 1.

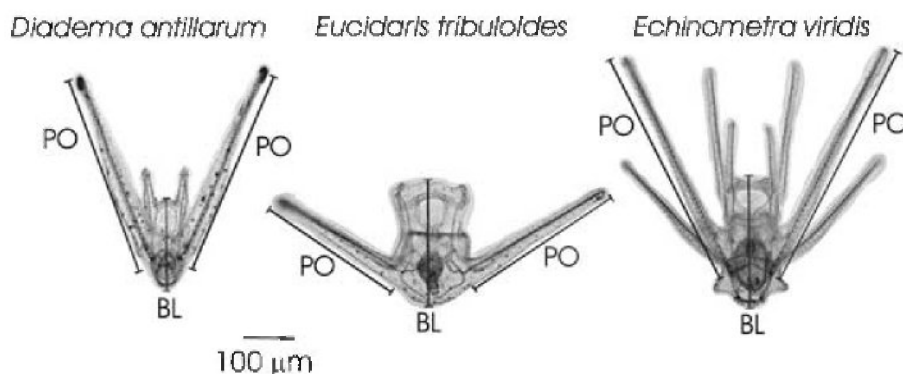
Fertilized embryos and larvae of each species were reared in one of two replicated food environments (5 and 1 algal cells/ $\mu\text{l}$ ). Each food level was then replicated among three cultures. Each larval culture was fed the unicellular alga *Dunaliella tertiolecta* (UTEX Algal Supply, Austin, TX) daily, starting at 48 h (all ages reported are postfertilization). All cultures were reared in 0.45- $\mu\text{m}$  filtered seawater in 1-l plastic tri-pour beakers at densities of 1 larva per mL and water was changed every day. The cultures were maintained in a recirculating water bath held at 28°C and were continually stirred at approximately 10 strokes per min with acrylic paddles to homogenize food and to keep larvae in suspension (Strathmann, 1987). *Dunaliella tertiolecta* was cultured at room temperature in microwaved 0.45- $\mu\text{m}$  filtered seawater enriched with a modified Guillard's f/2 medium (Florida Aqua Farms, Inc., Dade City, FL). Algae were separated from the growth medium by centrifugation and then resuspended in fresh 0.45- $\mu\text{m}$  filtered seawater before use.

## MEASURES OF PHENOTYPE

On days 2, 3, 4, 5, 6, 8, and 10 approximately 10 larvae were removed from each culture. Larvae were placed on a glass slide, immobilized with a dilute (<10%) solution of buffered formalin in seawater, and covered with a glass cover slip raised on clay feet. Three-dimensional Cartesian coordinates were recorded of multiple morphological features for five larvae from each culture (Fig. 2). These landmarks included the tip and base of each anterolateral, postoral, posterolateral, and posterodorsal arm rod, the posterior tip of the larva, and the tip of the oral hood (i.e., the midpoint of the soft-tissue that stretches between the pair of anterolateral arms). To collect data from each larva, I used a camera lucida (drawing tube) and a digitizing tablet (Hyperpen 12000U, Aiptek Inc., Irvine, CA) to capture  $x$  and  $y$  coordinates of morphological landmarks. Simultaneously, I obtained  $z$  coordinates from a rotary encoder (U.S. Digital, Vancouver, WA) 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 and body lengths (measured in millimeters) for each larva. Because the postoral arms were the first arm pair to develop in all species used in this study, and were the most prominent arms at all developmental stages when I collected measurements, my analysis focuses on plasticity in their summed length (sum of postoral arms).

## STATISTICAL ANALYSIS

Analysis of variance (PROC MIXED: SAS Institute, Cary, NC) tests were conducted (1) across all species and geminate pairs ("ocean analysis") and (2) for each geminate species pairing



**Figure 2.** Low-fed *Diadema antillarum*, *Echinometra viridis*, and *Eucidaris thouarsi* larvae at 10 days of development postfertilization. Morphological characters that I measured on days 2, 3, 4, 5, 6, 8, and 10: PO, postoral arm; BL, body length at midline. All larvae are displayed at the same magnification; scale bar represents 100 microns.

(“paired species analyses”), using the natural log-corrected sum of the postoral arm lengths (arm length) as the response variable in all statistical models. For the ocean analysis, I tested for the effect of variation among ocean, genus, family, day of development (day), food level (food), and culture replicate (culture) on arm length. The statistical model included the following interaction terms: ocean with food, ocean with day, day with food, ocean with genus, genus with food, and the three-way interactions of ocean by day by food and ocean by genus by food. Ocean, genus, day, food, and the interaction terms were coded as fixed effects and family and culture as random effects. The factor culture was nested within ocean, family, and food.

For the paired species analyses, I tested for the effect of variation among species, family, day, food, and culture on arm length. The model included terms to account for variation due to the interaction of species with food, day with food, species with day, and the three-way interaction of species by day by food. Species, day, food, and the interaction terms were coded as fixed effects and family and culture as random effects. The factor culture was nested within species, family, and food. The following paired species analyses were conducted: *D. antillarum*–*D. mexicanum*; *Ec. vanbrunti*–*Ec. lucunter*; *Ec. vanbrunti*–*Ec. viridis*; and *Eu. thouarsi*–*Eu. tribuloides*.

In both the ocean analysis and the paired species analyses, 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). Degrees of freedom were calculated using the DDFM = BW (Between-Within) option in PROC MIXED. Natural log-corrected midline body length (body length) was included in all models as a quantitative covariate. I compared models both with and without the body length interaction terms and used the models (no interaction terms) that provided the better fit to the data using Akaike’s information criteria (AIC) (Littell et al. 1996).

### TEST OF FOOD LIMITATION

I conducted a second experiment to test the effects on larval development of food levels lower than 1 algal cell/ $\mu$ l. Adult *Ec. vanbrunti* and *Ec. viridis* sea urchins were collected in August 2006 from the same respective Pacific and Caribbean field sites as described for the 2005 study (see above). Transportation of adult urchins to Naos and their maintenance in flow-through seawater aquaria were similar for this experiment. Gametes were obtained from adult urchins by peristomial injection of 0.5 M KCl. Fertilizations were conducted by combining eggs from seven female with sperm from four male *Ec. viridis*, and in a separate container, eggs from two female with sperm from four male *Ec. vanbrunti*. Initial mean ( $\pm$  1 SE) egg diameters (means of 10 eggs each) for *Ec. vanbrunti* females were 70.17 ( $\pm$ 0.45) and for *Ec. viridis* females were 86.53 ( $\pm$ 0.35). Assuming a sphere, mean egg volumes ( $\pm$  1 SE) were 0.18 ( $\pm$ 0.00) and 0.34 ( $\pm$ 0.00) nL, respectively.

Fertilized embryos and larvae of each species were reared in one of five replicated food environments (High—5, Low—1, Half—0.5, Limit—0.1, and Zero—0 algal cells/ $\mu$ l). Each food level was then replicated among three cultures. Larval cultures were fed the unicellular alga *D. tertiolecta* (UTEX Algal Supply, Austin, TX) daily, starting at 48 h. All cultures were reared in 0.45- $\mu$ m filtered seawater in 1-l plastic tri-pour beakers at densities of 1 larva per mL and water was changed every day. Larval cultures were maintained in the same manner (i.e., placed in a recirculating water bath held at 28°C, etc.), and *D. tertiolecta* cultures were reared and dispensed to larvae as described for the 2005 experiment.

For this second experiment, measures of phenotype were collected on days 2, 3, 4, 6, and 8. Analyses of variance (PROC MIXED: SAS Institute, Cary, NC) test were conducted between the two species and five food levels using the natural log-corrected sum of the postoral arm lengths (arm length) and/or midline body length (body length) as the response variables in the statistical

models. I tested for the effect of variation among species, day, food, and culture on arm length in one analysis of variance (ANOVA) and on body length in a separate ANOVA. The statistical models included terms to account for variation due to the interaction of species with food, day with food, species with day and the three-way interaction of species by day by food. Species, day, food, and the interaction terms were coded as fixed effects and culture as a random effect. The factor culture was nested within species and food. An analysis of variance (PROC MIXED) was also conducted between the two species and only the high and zero food levels using arm length as the response variable. Body length was included in the models testing for differences in arm length as a known quantitative covariate. In all statistical models for the food limitation experiment, day was coded as a repeated measure with culture as the subject and the covariance structure of the R matrix was specified as Compound Symmetry (CS). Degrees of freedom were calculated using the DDFM = BW (Between-Within) option in PROC MIXED.

### Results

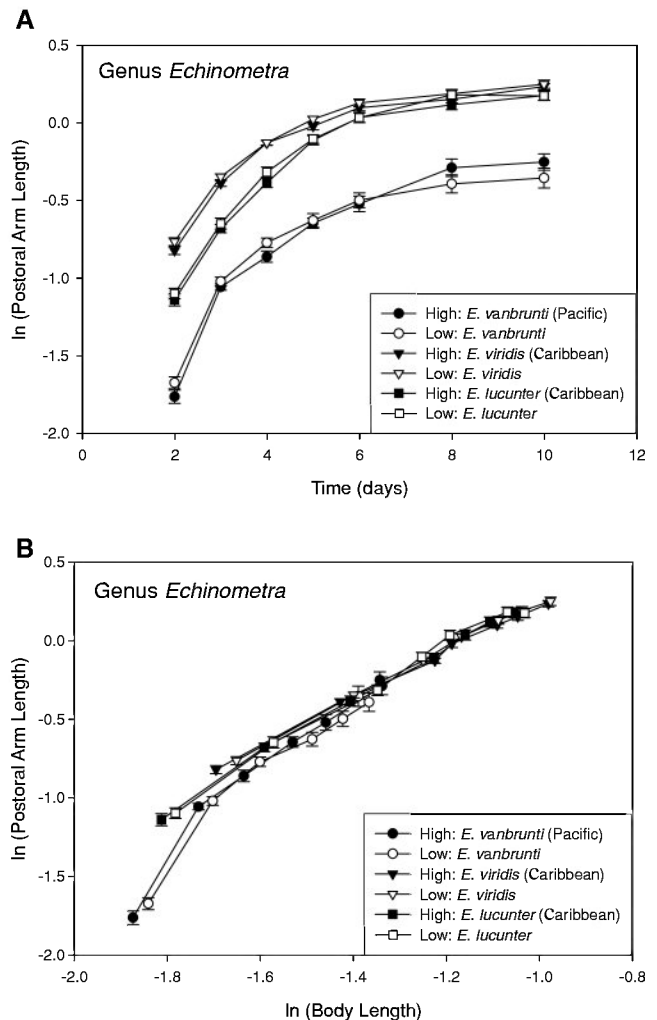
ANOVA among larvae from all Pacific and all Caribbean species (the ocean analysis) fed High (5 algal cells/ $\mu$ l) or Low (1 algal cell/ $\mu$ l) food levels in the 2005 experiment using arm length as the response variable detected significant effects due to genus, ocean, day, body length, and the interactions of ocean with day and ocean with genus (Table 2). There was no effect due to food, the interactions of ocean with food, day with food, genus with food, or to the three-way interactions of ocean by day by food and ocean by genus by food. The least square mean ( $\pm 1$  SE; units = ln mm) estimate of arm length corrected for body length was

**Table 2.** Analysis of Variance (ANOVA) results for all Caribbean versus all Pacific species larvae. Dependent variable is the natural log of the sum of postoral arm lengths. Natural log-corrected midline body length was included in the model as a known quantitative covariate.

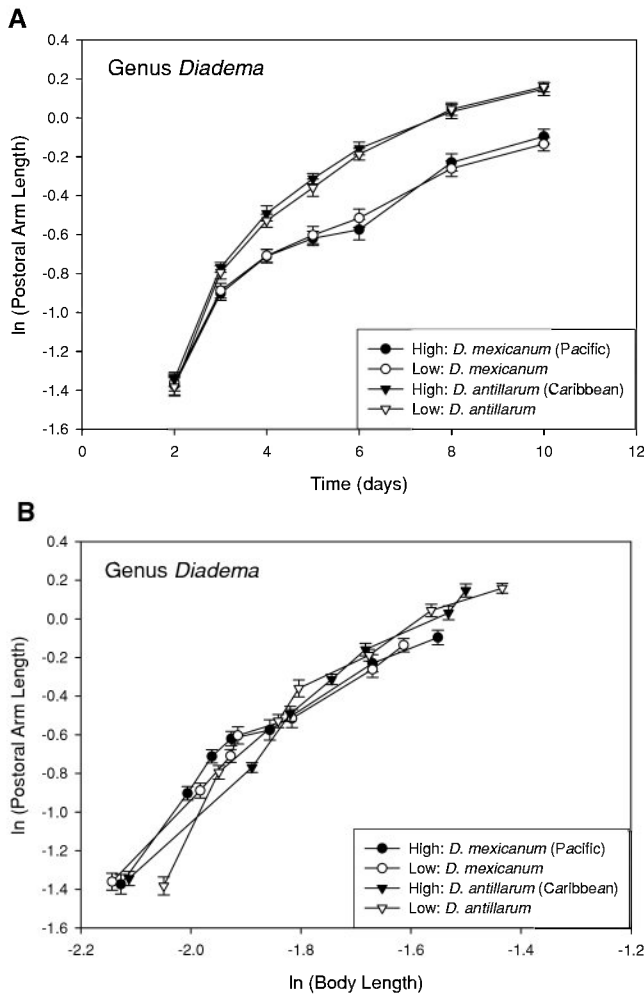
Effect	df: N, D	F Value	Pr>F
Genus	2, 40	1247.93	<0.0001
Ocean	1, 38	9.92	0.0032
Day	6, 228	467.05	<0.0001
Food	1, 38	0.33	0.5686
Ocean $\times$ Food	1, 38	0.57	0.4556
Ocean $\times$ Day	6, 228	10.90	<0.0001
Day $\times$ Food	6, 228	1.18	0.3156
Ocean $\times$ Genus	2, 40	397.93	<0.0001
Genus $\times$ Food	2, 40	3.13	0.0547
Ocean $\times$ Day $\times$ Food	6, 228	0.51	0.8025
Ocean $\times$ Genus $\times$ Food	2, 40	3.01	0.0608
ln (body length)	1, 3347	732.62	<0.0001

$-0.5542 \pm 0.062$  ( $t_{38} = -8.96$ ;  $P < 0.0001$ ) for the Caribbean and  $-0.6314 \pm 0.063$  ( $t_{38} = -10.03$ ;  $P < 0.0001$ ) for the Pacific.

Longer absolute arm lengths were expressed by Caribbean species of the genera *Echinometra* (Fig. 3A) and *Diadema* (Fig. 4A) over all developmental days postfertilization as compared to their Pacific geminate counterparts. Conversely, longer absolute arm lengths were expressed by the Pacific *Eu. thourarsi* through Day 6 (Fig. 5A); between Day 6 and Day 10, the Caribbean *Eu. tribuloides* exhibited an increase in absolute arm length (Fig. 5A). Longer relative arm to body lengths were expressed

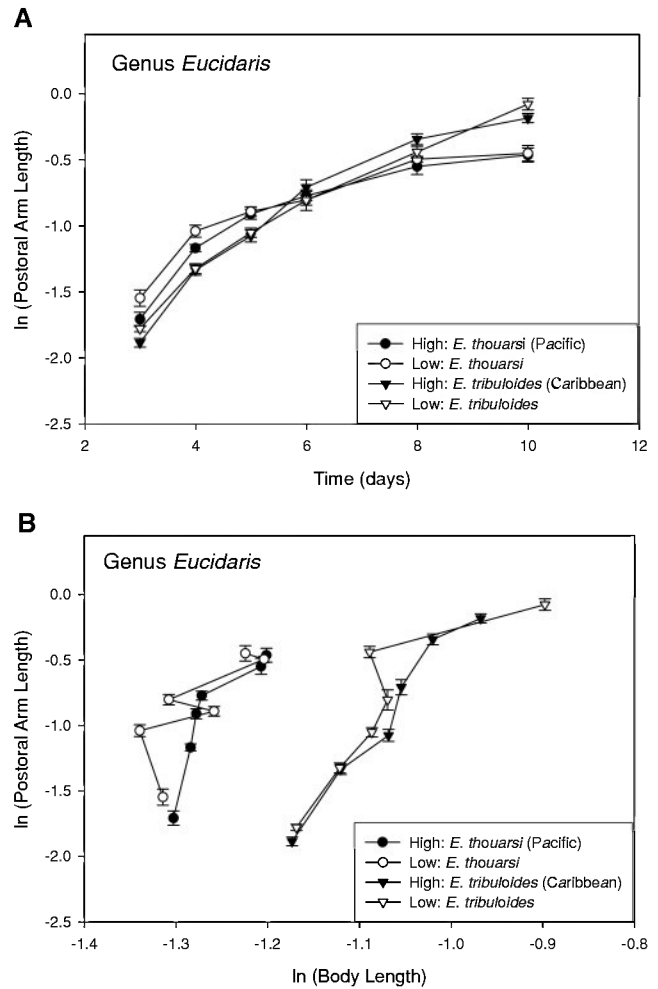


**Figure 3.** (A) Mean ( $\pm 1$ SE) natural log-corrected summed length of Postoral arms for High-food (filled symbols) and Low-food (open symbols) larvae from the genus *Echinometra* over time. (B) Mean ( $\pm 1$ SE) natural log-corrected summed length of Postoral arms versus mean ( $\pm 1$ SE) natural log corrected Body Length at midline for High-food (filled symbols) and Low-food (open symbols) larvae from the genus *Echinometra*. In both A and B, circle symbols indicate values for *Echinometra vanbrunti*, triangle symbols indicate values for *Echinometra viridis*, and square symbols indicate values for *Echinometra lucunter*. Units are ln millimeters.



**Figure 4.** (A) Mean ( $\pm 1SE$ ) natural log corrected summed length of Postoral arms for High-food (filled symbols) and Low-food (open symbols) larvae from the genus *Diadema* over time. (B) Mean ( $\pm 1SE$ ) natural log-corrected summed length of Postoral arms versus mean ( $\pm 1SE$ ) natural log-corrected Body Length at midline for High-food (filled symbols) and Low-food (open symbols) larvae from the genus *Diadema*. In both A and B, circle symbols indicate values for *Diadema mexicanum* and triangle symbols indicate values for *Diadema antillarum*. Units are ln millimeters.

by both Caribbean *Echinometra* species (Fig. 3B) compared to the Pacific species over all days. A similar pattern was exhibited by the Caribbean *D. antillarum* as compared to the Pacific *D. mexicanum* after approximately 4–5 days of development (Fig. 4B). Trajectories of arm to body length for both *Eucidaris* species indicate that larvae of the Caribbean *Eu. tribuloides* have larger bodies than the Pacific *Eu. thouarsi* throughout the period of measurement (Fig. 5B). The distinct arm length relative to body length growth patterns expressed by both *Eucidaris* species, as compared to the other species used in this study (Figs. 3B, 4B, and 5B), may reflect the fact that over the time frame of this study neither of these species projected distinct anterolateral arms with



**Figure 5.** (A) Mean ( $\pm 1SE$ ) natural log-corrected summed length of Postoral arms for High-food (filled symbols) and Low-food (open symbols) larvae from the genus *Eucidaris* over time. (B) Mean ( $\pm 1SE$ ) natural log-corrected summed length of Postoral arms versus mean ( $\pm 1SE$ ) natural log-corrected Body Length at midline for High-food (filled symbols) and Low-food (open symbols) larvae from the genus *Eucidaris*. In both (A) and (B), circle symbols indicate values for *Eucidaris thouarsi* and triangle symbols indicate values for *Eucidaris tribuloides*. Units are ln millimeters.

rigid structural elements from the oral hood (the soft-tissue area between the anterolateral arms; see Fig. 2). The anterolateral arms help to lengthen and support the larval bodies in most species, providing for more accurate linear body measurements. The bodies of *Eucidaris* larvae tended to curl inwards as they grew larger, even while alive and before slide preparation procedures were conducted. This slight curling of the body affected measurements of *Eucidaris* sp. body lengths, making them artificially shorter.

Paired-species ANOVAs using arm to body length ratio as the response variable between larvae fed High (5 algal cells/ $\mu$ l) or Low (1 algal cell/ $\mu$ l) food levels in the 2005 experiment support the visual interpretations of Figures 3–5 and detected the

**Table 3.** Analysis of Variance (ANOVA) results for larvae from geminate species pairs, that is, separate tests between a given Caribbean species versus its Pacific species geminate. Dependent variable in each model is the natural log of the sum of postoral arm lengths. Natural log-corrected midline body length was included in each model as a known quantitative covariate. Effect abbreviations: S, species; D, day; F, food; lnBL, natural log of body length. Test abbreviations: df, degrees of freedom; n, numerator; d, denominator.

Effect	Species pairs tested: Analysis of Variance											
	<i>E. viridis</i> vs. <i>E. vanbrunti</i>			<i>E. lucunter</i> vs. <i>E. vanbrunti</i>			<i>D. antillarum</i> vs. <i>D. mexicanum</i>			<i>E. tribuloides</i> vs. <i>E. thouarsi</i>		
	df: n, d	F value	Pr>F	df: n, d	F value	Pr>F	df: n, d	F value	Pr>F	df: n, d	F value	Pr>F
S	1, 32	153.41	<0.0001	1, 38	105.35	<0.0001	1, 20	215.43	<0.0001	1, 20	17.13	0.0005
D	6, 185	153.97	<0.0001	6, 221	197.03	<0.0001	6, 109	312.59	<0.0001	5, 77	466.66	<0.0001
F	1, 32	0.12	0.7353	1, 38	0.08	0.7723	1, 20	0.24	0.6319	1, 20	1.01	0.3267
S × F	1, 32	0.22	0.6395	1, 38	0.19	0.6660	1, 20	0.67	0.4218	1, 20	0.60	0.4470
S × D	6, 185	58.32	<0.0001	6, 221	15.40	<0.0001	6, 109	10.24	<0.0001	5, 77	21.50	<0.0001
D × F	6, 185	1.65	0.1365	6, 221	1.90	0.0813	6, 109	0.16	0.9864	5, 77	1.95	0.0960
S × D × F	6, 185	1.71	0.1201	6, 221	1.59	0.1512	6, 109	0.59	0.7412	5, 77	0.76	0.5819
lnBL	1, 1151	1234.13	<0.0001	1, 1351	1104.55	<0.0001	1, 722	199.09	<0.0001	1, 558	43.94	<0.0001

following patterns (see Table 3 for values). The ANOVAs between larvae of *Ec. vanbrunti* and *Ec. viridis*, *Ec. vanbrunti* and *Ec. lucunter*, *D. mexicanum* and *D. antillarum*, and *Eu. thouarsi* and *Eu. tribuloides* detected significant effects of species, day, body length, and species with day within each analysis. In each analysis, there was no effect due to food, species with food, day with food, or to the three-way interaction of species by day by food. Visual inspection of the arm to body length trajectories for each species support this result (Figs. 3B, 4B, and 5B). The least square mean ( $\pm 1$  SE : units = ln mm) estimates of arm length to body length ratio for species from each analysis are given in Table 4.

#### FOOD LIMITATION ANALYSIS

Caribbean *Ec. viridis* larvae did not respond significantly to limiting food conditions; arm to body length trajectories are comparable across all five food treatments (Fig. 6A). The growth of larval arms relative to body in Pacific *Ec. vanbrunti* larvae was affected

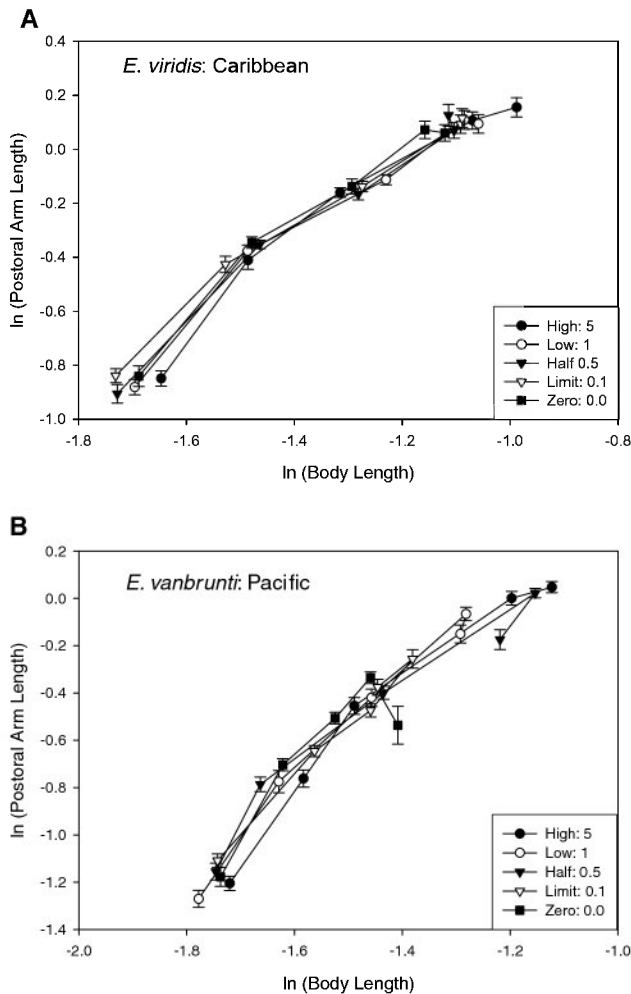
by food concentrations lower than 1.0 algal cell/ $\mu$ l; trajectories for the lower food treatments do not extend as far as for the higher food treatments (Fig. 6B). This result suggests that food is limiting for Pacific species but not for Caribbean species. In support of this finding, the ANOVA between *Ec. vanbrunti* and *Ec. viridis* larvae fed High (5 algal cells/ $\mu$ l), Low (1 algal cell/ $\mu$ l), Half (0.5 algal cell/ $\mu$ l), Limit (0.1 algal cell/ $\mu$ l), or Zero (0 algal cell/ $\mu$ l) food levels in the 2006 experiment using body length as the response variable detected significant effects of species ( $F_{1,20} = 165.19$ ,  $P < 0.0001$ ), day ( $F_{4,74} = 572.49$ ,  $P < 0.0001$ ), food ( $F_{1,20} = 6.50$ ,  $P = 0.0016$ ), species with day ( $F_{4,74} = 13.70$ ,  $P < 0.0001$ ), day with food ( $F_{16,74} = 4.76$ ,  $P < 0.0001$ ), and the three-way interaction of species by day by food ( $F_{16,74} = 2.68$ ,  $P = 0.0022$ ). There was no effect due to species with food ( $F_{4,20} = 2.30$ ,  $P = 0.0938$ ).

Low food levels did not induce statistically significant phenotypically plastic responses in larvae from any food treatment lower than or equal to 1.0 algal cell/ $\mu$ l; there was no effect due

**Table 4.** Least square mean estimates (units=ln mm) from each of the analyses of variance (ANOVAs) between oceans (Table 2) and geminate species pairs (see Table 3).

Analysis	Effect	Ocean/Species	Estimate ( $\pm 1$ SE)	df	t Value	Pr> t
Ocean Analysis	Ocean	Caribbean	-0.5542 $\pm$ 0.062	38	-8.96	<0.0001
		Pacific	-0.6314 $\pm$ 0.063	38	-10.03	<0.0001
Paired Species Analysis	Species	<i>Ec. viridis</i>	-0.2575 ( $\pm 0.056$ )	32	-4.59	<0.0001
		<i>Ec. vanbrunti</i>	-0.5902 ( $\pm 0.056$ )	32	-10.51	<0.0001
	Species	<i>Ec. lucunter</i>	-0.3552 ( $\pm 0.066$ )	38	-5.35	<0.0001
		<i>Ec. vanbrunti</i>	-0.5846 ( $\pm 0.067$ )	38	-8.68	<0.0001
	Species	<i>D. antillarum</i>	-0.3468 ( $\pm 0.1219$ )	20	-2.85	0.0010
		<i>D. mexicanum</i>	-0.6222 ( $\pm 0.1209$ )	20	-5.15	<0.0001
Species	<i>Eu. tribuloides</i>	-0.9604 ( $\pm 0.046$ )	20	-20.70	<0.0001	
	<i>Eu. thouarsi</i>	-0.7705 ( $\pm 0.058$ )	20	-13.27	<0.0001	





**Figure 6.** Mean ( $\pm 1SE$ ) natural log-corrected summed length of Postoral arms for High-food (5 algal cells/ $\mu\text{l}$ : filled circle symbols), Low-food (1 algal cell/ $\mu\text{l}$ : open circle symbols), Half-food (0.5 algal cell/ $\mu\text{l}$ : filled triangle symbols), Limit-food (0.1 algal cell/ $\mu\text{l}$ : open triangle symbols), and Zero-food (0.0 algal cell/ $\mu\text{l}$ : filled square symbols) larvae versus mean ( $\pm 1SE$ ) natural log-corrected Body Length at midline. In (A), values for Caribbean *Echinometra viridis* larvae are indicated. In (B), values for Pacific *Echinometra vanbrunti* larvae are indicated. Larvae from both species were reared in these food treatments during the subsequent food-limitation experiment conducted in 2006. Units are ln millimeters.

to food in the ANOVA using arm length as the response variable. This ANOVA was structured the same as the ANOVA for body length described above, although including body length as a quantitative covariate, and detected significant effects of species, day, body length, and the interactions of species with day, species with food, day with food, and the three-way interaction of species by day by food (see Table 5). A smaller ANOVA between *Ec. vanbrunti* and *Ec. viridis* larvae fed High (5 algal cells/ $\mu\text{l}$ ) or Zero (0 algal cell/ $\mu\text{l}$ ) food levels using arm length as the response variable and body length as a covariate detected significant effects of

**Table 5.** Analysis of Variance (ANOVA) results for Caribbean *Echinometra viridis* versus Pacific *Echinometra vanbrunti* larvae fed either High (5.0 algal cells/ $\mu\text{l}$ ), Low (1.0 algal cell/ $\mu\text{l}$ ), Solow (0.5 algal cell/ $\mu\text{l}$ ), Limit (0.1 algal cell/ $\mu\text{l}$ ), or Zero (0.0 algal cell/ $\mu\text{l}$ ) food levels. Dependent variable is the natural log of the sum of postoral arm lengths. Natural log-corrected midline body length was included in the model as a known quantitative covariate.

Effect	df: N, D	F value	Pr>F
Species	1, 20	306.12	<0.0001
Day	4, 74	320.78	<0.0001
Food	4, 20	1.84	0.1605
Species $\times$ Food	4, 20	3.69	0.0209
Species $\times$ Day	4, 74	21.34	<0.0001
Day $\times$ Food	16, 74	6.24	<0.0001
Species $\times$ Day $\times$ Food	16, 74	4.60	<0.0001
ln (Body Length)	1, 649	308.07	<0.0001

species ( $F_{1,8} = 78.97$ ,  $P < 0.0001$ ), day ( $F_{4,29} = 147.66$ ,  $P < 0.0001$ ), body length ( $F_{1,256} = 79.42$ ,  $P < 0.0001$ ), the interactions of species with day ( $F_{4,29} = 5.69$ ,  $P = 0.0017$ ), species with food ( $F_{1,8} = 6.50$ ,  $P = 0.0342$ ), day with food ( $F_{4,29} = 16.77$ ,  $P < 0.0001$ ), and the three-way interaction of species by day by food ( $F_{4,29} = 9.84$ ,  $P < 0.0001$ ). There was no effect due to food ( $F_{1,8} = 3.89$ ,  $P = 0.0842$ ).

## Discussion

In the introduction, I posed three hypotheses regarding how differences in the heterogeneity of phytoplankton food levels may influence the evolution and expression of larval arm length. The results presented in this study do not support the two plasticity hypotheses; however they do support the constant differences hypothesis.

### CONSTANT DIFFERENCES

The results from the “ocean” analysis (Tables 2 and 4) indicated that larvae from the Caribbean had longer arms relative to body length than larvae from the Pacific. Similarly, the results from the “paired species” analyses of variance indicated that larvae from Caribbean species in the genera *Diadema* and *Echinometra* had longer arms relative to body length than their Pacific geminate counterparts. There were significant effects due to species on postoral arm length corrected for body length in each of these “paired species” analyses of variance (Table 3) and the least square mean estimates were greater for each Caribbean species than for each Pacific species, within each respective comparison (Table 4). However, the result from the “paired species” analysis of variance between the Caribbean and Pacific *Eucidaris* species indicated the opposite; *Eu. thouarsi* from the Pacific had longer arms relative to body length than *Eu. tribuloides* from the Caribbean (Tables 3

and 4). The result for *Eucidaris* may reflect the fact that only one *Eu. thouarsi* family was used in the analysis, as opposed to three *Eu. tribuloides* families. I had considerable difficulty obtaining mature gametes from *E. thouarsi* during the time I conducted the experiments (mid-June through early September in 2005 and August of 2006); I injected over 120 individuals with 0.5 M KCl to induce spawning and obtained mature gametes from only one male and one female.

The significant difference in relative larval arm length detected across ocean basins when incorporating all species (the large “ocean” analysis of variance) must be interpreted carefully (Table 2). Analysis of variance incorporates the magnitude as well as the direction of differences between categories; therefore it is possible that a large, directional difference in one (or more) geminate species pairings could have led to the overall significant difference detected across ocean basins. I conducted the paired species analyses to account for this possibility and to aid in the interpretation of this result. Note that three of the four species pairs I examined exhibited the same pattern: longer relative arm lengths for the Caribbean species in each separate pairing of *Echinometra* sp. and with the *Diadema* (Table 3). *Eucidaris* was the only genus that showed the opposite pattern, perhaps influenced in part due to the body length measurement issue mentioned in Results (above). A signed-rank test would aid in the interpretation of the overall pattern, however there are not enough easily collectable echinoid geminate species pairs with feeding larvae (i.e., at least six independent pairs) in this system to perform this type of test.

The results from the “ocean” analysis and the “paired species” analyses for *Diadema* and *Echinometra* support the Constant Differences Hypothesis; larvae evolving in the constantly low phytoplankton food levels of the western Caribbean grew longer arms relative to body length than larvae evolving in the variable food levels of the eastern Pacific. In an environment with little to no heterogeneity in food resources, as characterized by the Caribbean, an appropriate evolutionary strategy for resource acquisition may be to express constantly long arms relative to body length. Expressing a constant long arm phenotype may produce a better return (in terms of exogenous energy acquisition) on the investment in long arms (in terms of materials to produce and metabolism to maintain) than expressing a plastic arm length phenotype. If phenotypic plasticity confers a benefit in heterogeneous environments, as characterized by the Pacific, then there may be no benefit from plasticity of arm length for larvae evolving in the homogeneous environment of the Caribbean; a cost of plasticity may also constrain the expression of arm length plasticity in a homogeneous environment.

## PHENOTYPIC PLASTICITY

Contrary to the published findings of several researchers using various, diverse echinoid species (Boidron-Metairon 1988; Hart

and Scheibling 1988; Strathmann et al. 1992; Hart and Strathmann 1994; Sewell et al. 2004; Reitzel and Heyland 2007), none of the species I reared in this study exhibited phenotypic plasticity of larval arm length. There was no significant effect due to food on postlarval arm length corrected for body length detected in either the “ocean” analysis of variance (Table 2) or any of the “paired species” analyses of variance (Table 3). This surprising finding begs the question as to why there was no, or minimal, that is, no statistically significant, phenotypic plasticity of larval arm length exhibited by any of the seven species reared in this study?

The simplest explanation for this result may be that the low food level I used (1.0 algal cell/ $\mu$ l) was not low enough to induce a phenotypically plastic response. In other words, this food level may not have been representative of a food limiting condition for these larvae; however, this food level falls within the range of “low” food levels used in other studies demonstrating arm length plasticity in larval echinoids (2 algal cells/ $\mu$ l: Miner 2005; Reitzel and Heyland 2007;  $\sim$ 1.3 algal cells/ $\mu$ l: Boidron-Metairon 1988; 0.6 algal cells/ $\mu$ l: Sewell et al. 2004; 0.5 algal cells/ $\mu$ l: McAlister 2007; 0.3 cells/ $\mu$ l: Hart and Strathmann 1994). I chose 1.0 algal cell/ $\mu$ l as a low food treatment because lower food levels have been demonstrated to result in stalled larval development in some invertebrate species (Pechenik et al. 1984; Eckert 1995; Herrera et al. 1996). The results from the food limitation experiment I conducted in 2006 (the second experiment described above) using *Ec. viridis* and *Ec. vanbrunti* indicated that these species did not express plasticity of larval arm length at food treatments lower than 1.0 algal cell/ $\mu$ l; there was no significant effect due to food on postlarval arm length corrected for body length detected by the analysis of variance among the five different food treatments (Table 5). There was no effect on larval body length with decreasing food ration on Caribbean *Ec. viridis* (see Fig. 6A). However, the two lowest food rations (0.1 algal cell/ $\mu$ l and 0.0 algal cell/ $\mu$ l) did limit development of larval body length in Pacific *Ec. vanbrunti* (see Fig. 6B). The results from the food limitation experiment suggest that the lack of measurable levels of phenotypic plasticity of arm length within this system (i.e., all of the species used in the 2005 experiment) is a true finding. As mentioned, these results run counter to the published findings of plasticity from multiple other echinoderm species (Boidron-Metairon 1988; Hart and Scheibling, 1988; Strathmann et al. 1992; Hart and Strathmann 1994; Sewell et al. 2004; Reitzel and Heyland 2007). These results do not preclude the fact that there may be additional species that are similarly nonplastic; other negative findings may have no record of publication.

None of the species examined in this study demonstrated phenotypic plasticity of larval arm length; the results indicated that there are no differences in degree of plasticity across all species and do not support either the plasticity or differential plasticity hypotheses. The results do suggest that despite the well

documented and historical differences in productivity between the eastern Caribbean and western Pacific (Glynn 1982; Keigwin 1982), there may be less difference in the variability of food resources between these environments, on a scale that is relevant to larvae. Additionally, when differences in egg size across the geminate pairs are considered, the lack of plasticity in these species suggests that selection may have acted on other life-history characteristics to account for differences in the levels of exogenous phytoplankton food. Alternatively, the possibility exists that a different measure of plasticity (e.g., arm length relative to juvenile rudiment length) might detect significant plasticity in response to food and/or divergence in plasticity across the Isthmus. This comparison remains for future studies because the larvae cultured in this study were only reared for 10 days, that is, not long enough for all species in each food treatment to develop juvenile rudiments consistently. Although the negative finding of a lack of plasticity is the main strength of this study, interpreting this result, and the interesting trends in relative arm length, as evolutionary responses must be tempered, however, by the fact that we do not know, and cannot determine in this system, what the ancestral conditions were with regard to plasticity, relative arm length, or egg size. I discuss these possibilities, concerns, and the collective results of my experiments below.

#### **PHYLOGENETIC CONSIDERATIONS: IMPLICATIONS FOR THE EVOLUTION OF PLASTICITY**

Recent phylogenetic evidence for echinoderm classes supports two plausible phylogenies: echinoids + holothuroids as a sister group to asteroids + ophiuroids and/or echinoids + holothuroids as a sister group to ophiuroids, which combined form a sister group to asteroids (Littlewood et al. 1997; Janies 2001). Within the echinoids, this study examines plasticity in species representing the oldest lineage (Order Cidaroida) and second oldest lineage (Order Diadematoidea) that still have feeding larvae and in a more recent lineage (Order Echinoida) (Giese et al. 1991). Although plasticity has been observed in echinoid species from other recent lineages (reviewed in Sewell et al. 2004), no other studies have examined any taxa in the two oldest lineages. The lack of plasticity in the *Eucidaris* sp. and *Diadema* sp. examined in this study suggests that phenotypic plasticity may have only evolved in more derived echinoids, and thus a lack of plasticity may be the ancestral condition. Alternatively, data from studies on asteroids (George 1994, 1999) and ophiuroids (Podolsky and McAlister 2005) indicate that the species examined from these groups exhibit phenotypic plasticity of feeding structures, whereas data from one holothuroid species showed no difference in body form between well-fed and poorly fed larvae (Strathmann et al. 1994), which may indicate that plasticity is ancestral. The number of species examined for groups other than the echinoids are extremely limited however

and do not provide strong support for either plasticity or a lack of plasticity as the ancestral condition.

#### **ENVIRONMENTAL VARIATION IN RESOURCE LEVELS: LOCAL AND LATITUDINAL CONSIDERATIONS**

Tropical coastal marine ecosystems are commonly oligotrophic with patchy food resources (Koblentz-Mishke et al. 1970; Mackas et al. 1985) for planktonic larvae. Alternatively, levels of primary productivity in temperate coastal ecosystems can cycle between low levels in winter and large peaks during spring and summer algal blooms (Lalli and Parsons 1993). Values of chlorophyll *a* concentration, a measure of phytoplankton concentration, for coastal waters suggest that larvae, regardless of ecosystem, are usually food-limited to some degree (Paulay et al. 1985), although comparison among ecosystems is crude because of different assemblages of algal species and lack of information about natural dietary preferences. Published chlorophyll *a* concentrations are less and in some areas approximately one order of magnitude lower in tropical (0.01 to 0.35  $\mu\text{g/L}$  for Moorea, Society Islands; Ricard, 1981; 0.19 to 0.52  $\mu\text{g/L}$  in waters of the Great Barrier Reef; Lucas, 1982; 0.59  $\mu\text{g/L}$  during the rainy season and 1.48 during the dry season of upwelling in the Bay of Panama, and 0.41 during the rainy season and 0.36 during the dry season at San Blas Point in the Caribbean; calculated from values reported in  $\text{mg per m}^3$  by D'Croz and Robertson 1997) than in temperate ecosystems (<1  $\mu\text{g/L}$  in winter to >15  $\mu\text{g/L}$  in spring blooms off the Washington and Oregon coasts; Richards 1950; Anderson 1964; Harrison et al. 1983; and 1.3 to 3.8  $\mu\text{g/L}$  in August in Long Island Sound; Whitley and Wirick 1983). These values suggest that larvae of tropical species may be food-limited to a greater degree than larvae of temperate species.

Faced with constant low food levels, the tropical planktrophic larvae from the Caribbean species examined in this study may have evolved to express a constant long larval arm length phenotype instead of plasticity of arm length. In tropical habitats with widespread resource patchiness, expressing a constant long arm length phenotype likely increases the food gathering capability of a given larva. Conversely, plasticity of arm length may be an evolutionary strategy that results in greater food gathering capability for larvae in temperate habitats. Matched against the patterns of ecosystem productivity, plasticity of arm length in pluteus larvae has been demonstrated primarily in temperate species. Some of the highest magnitudes of larval arm length plasticity are recorded for species from cold temperate waters (Boidron-Metairon 1988; Hart and Scheibling 1988; Sewell et al. 2004) whereas some of the lowest are recorded for species from warm tropical or subtropical waters (Boidron-Metairon 1988; Eckert 1995; Podolsky and McAlister 2005). There are certainly exceptions to the observation that the magnitude of plasticity varies with the productivity of an

ecosystem (e.g., Fenaux et al. 1994 detected plasticity in *Paracentrotus lividus* from nutrient poor Mediterranean waters), however the general pattern suggests that there may be a latitudinal gradient in phenotypic plasticity of larval feeding structures. Comparative studies of plasticity in multiple populations of species whose ranges span tropical and temperate ecosystems would help to discern this pattern. To my knowledge there are limited studies that compare plasticity among multiple populations (see Bertram and Strathmann 1998 for one example) of any species of marine invertebrate larvae.

Similar to the Caribbean species, the tropical Pacific species larvae in this system may not have evolved to express phenotypic plasticity because they may only experience low resource levels. Despite the well-documented annual heterogeneity of resource levels, some Pacific echinoids (e.g., *Diadema mexicanum* and *Ec. vanbrunti*) do not release their eggs during the period of the year with peak phytoplankton production (Lessios 1981). Consequently, larval settlement tends to occur before the period of seasonal upwelling (Lessios 1981). Reproduction during the off-season, with respect to phytoplankton production, suggests that these species may not be taking advantage of the higher resource levels during upwelling. However, timing their reproduction to avoid upwelling may mitigate the effects on duration of the larval period that could result from the lower water temperatures during upwelling (Thorson 1950; Glynn 1972; Hinegardner 1975; Lessios 1981). Species evolving in this habitat may time their reproduction and the duration of larval development to guarantee that larvae reach metamorphosis before upwelling. Furthermore, the upwelling period is characterized not only by high nutrient levels and lower water temperatures, but also by strong offshore transport (Smayda 1966; D'Croz and Robertson 1997). Larvae that are transported offshore may not be able to find suitable sites for postmetamorphic settlement (Lessios 1981). Reaching metamorphosis before upwelling would increase the probability that larval settlement occurs near shore.

Alternatively, there may be finer-scale, localized heterogeneity in food levels within each respective ocean basin. For example, intensity of upwelling varies along the Pacific coast of Central America (Wyrteki 1967; Legeckis 1985; McCreary et al. 1989). The adult urchins collected from the Pacific in this study came from the Bay of Panama, which has localized high levels of nutrient upwelling (D'Croz and O'Dea 2007). Other areas along the Pacific coast of Panama have lower levels of nutrient upwelling, for example, the Gulf of Chiriqui (D'Croz and O'Dea 2007). Within-ocean basin differences in the heterogeneity of food resources may affect the evolution of plasticity if there are high levels of larval exchange and genetic mixing among populations from different locales. Spatially heterogeneous environments with a high degree of patchiness are thought to select for the evolution of phenotypic plasticity (Levins 1968). However, in light of

the timing of reproduction, selection for small egg size in Pacific species and the constraints that low endogenous energetic resources may have on the expression of plasticity (see below), and the possibility of high levels of larval exchange among Pacific locales, selection may have favored a generalist fixed arm length strategy for resource acquisition, instead of a phenotypically plastic one.

#### **SELECTION ON LIFE-HISTORY CHARACTERS: THE CONFOUNDING ASPECT OF EGG SIZE**

A discussion of the evolution of phenotypic plasticity or phenotypic fixation of feeding structures in this system must consider the documented differences in egg size between Caribbean and Pacific species. Egg size has long been considered an important component of the life histories of marine organisms (Thorson 1950; Vance 1973; Christiansen and Fenchel 1979; Strathmann 1985; Jaekle 1995; Levitan 2000; Moran 2004; Allen 2005). In the Panamanian Isthmus system egg size is larger in many Caribbean species than in their Pacific geminates. Lessios (1990) has shown that members of geminate pair echinoids found in the western Caribbean have larger egg sizes than their eastern Pacific counterparts due to changes in productivity following the rise of the Isthmus of Panama. The results from the current study show the same pattern (see Table 1) in a subset of the species examined by Lessios (1990). A similar pattern has been demonstrated for bryozoans (Jackson and Herrera 1999) and arcid bivalves (Moran 2004). This pattern supports theoretical models that predict that the greater endogenous resources found in large eggs, which represent an increased maternal investment per ovum, evolve in response to a poor larval feeding environment, as found in the western Caribbean (Vance 1973; Lessios 1990; Levitan 2000). Conversely, small egg sizes in the eastern Pacific likely represent an evolutionary response to high levels of oceanic productivity (Lessios 1990; Moran 2004).

An investigation of the effects of egg size on the expression of phenotypic plasticity in the Panamanian echinoid system would be ideal. However, the arguments for the expression of plasticity as an evolutionary response to historical heterogeneity in food resource levels and to a reduction in egg size are confounded in the Panamanian system, that is, within each geminate pair, the species with smaller egg size inhabits the heterogeneous environment of the eastern Pacific. Results from a recent study I conducted using echinoid species in the genus *Strongylocentrotus* that differ in egg size (McAlister 2007) indicate, however, that large egg size is associated with the expression of greater degrees of phenotypic plasticity and of longer arm relative to body lengths than small egg size. In light of the results obtained in the current study, the expression of longer arm relative to body lengths in the Caribbean species may reflect the fact that these species develop from a larger egg than their Pacific counterparts. Caribbean species may

have obtained a greater benefit, in terms of fitness, by having experienced selection for larger initial endogenous energetic reserves, that is, larger egg size, than for phenotypic plasticity of exogenous food collection structures. The result of a greater degree of phenotypic plasticity in the larger-egged *Strongylocentrotus* species does not match the results obtained in the current study. This may be due to the fact that *Strongylocentrotus* is a temperate genus and the larger-egged species (*S. franciscanus*) examined by McAlister (2007) develops from an egg that is larger in size than any of the tropical species examined in the Panamanian system. Further research on larval growth and egg composition/quality using different populations of each Panamanian system species evolving in areas of different productivity may help to elucidate the patterns found in this study.

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