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The contribution of the facultative feeding period to echinoid larval development and size at metamorphosis: a comparative approach

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Abstract

Planktotrophic invertebrate larvae have two dissociable stages during development, a facultative feeding period, whose length is determined by the amount of maternal provisioning in the egg, and an obligate feeding period, whose length is determined by the quantity of exogenous energy needed to reach metamorphic competence. Here we set out to experimentally test the impact of feeding during the facultative feeding period at two food concentrations (limiting and nonlimiting) on larval development time and juvenile quality. We used two closely related subtropical sand dollar species that differ in the quantity of maternal investment for these comparisons: *Leodia sexiesperforata* (large egg, long facultative feeding period) and *Mellita tenuis* (small egg, short facultative feeding period). We found that feeding during the facultative period accelerates development to metamorphosis only in *M. tenuis* and only at the high food ration. Feeding during the facultative feeding period had no effect on development time for *M. tenuis* at a food limiting concentration and for *L. sexiesperforata* at either food concentration. Furthermore, we found feeding during the facultative period to significantly increase quantity of carbohydrates and lipids at metamorphosis only for *M. tenuis* in nonlimiting food concentration. Thus, our data reveal a two-fold benefit of the facultative feeding period for a poorly provisioned species under high food conditions but little effect on a well-provisioned species. We discuss our results in reference to McEdward's [McEdward, L.R., 1997. Reproductive strategies of marine benthic invertebrates revisited: facultative feeding by planktotrophic larvae. Am. Nat. 150, 48–72] facultative feeding model.

Keywords: Echinoid; Egg size; Facultative feeding; Larval development; Metamorphosis

1. Introduction

Marine invertebrate larvae have been historically classified into two distinct nutritional types: planktotrophic and lecithotrophic. Planktotrophic larvae must obtain exogenous food during development to reach

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metamorphic competence. Lecithotrophic larvae are provisioned with sufficient energy to complete development and metamorphose without exogenous food. The most important determinant of larval nutritional mode is, therefore, maternal provisioning per egg (reviewed by Levin and Bridges, 1995). In contrast to this dichotomy however, there is a continuum of maternal provisioning per egg within both nutritional modes (review Jaeckle, 1995; planktotrophs: Emlet et al., 1987; Herrera et al., 1996; lecithotrophs: Emlet et al., 1987; McEdward and Chia, 1991). Mathematical models have tested hypotheses for the evolutionary advantage and maintenance of different egg provisioning strategies within planktotrophs (fertilization: Levitan, 1993; Podolsky and Strathmann, 1996; mortality/growth rate: Christiansen and Fenchel, 1979; feeding strategies: McEdward, 1997; development time: Levitan, 2000). These models predict how selection should change the amount of energy mothers supply per egg by weighing the trade-offs among life history characters that correlate with maternal provisioning per egg.

The effect feeding has on planktotrophic larval development time, survivorship, and subsequent juvenile size have been studied extensively for a number of marine invertebrates (reviewed by Boidron-Metairon, 1995). Not surprisingly, larvae that are fed a higher concentration of food typically develop more quickly and metamorphose as larger juveniles when compared to sibling larvae fed less. Similarly, experiments comparing low and high quality food have shown that higher quality food results in shorter development time and/or larger juvenile size (Hinegardner, 1969; Lucas, 1982; Anger et al., 1986; McEdward and Herrera, 1999). The fitness advantages for shorter development time and increased juvenile size are likely to be sizeable. A reduction in development time reduces the probability of pelagic mortality, which may be extreme for larvae (15% day^{-1} reported by Rumrill, 1990; 16.4% day^{-1} reported by Lamare and Barker, 1999). Larger juvenile size can result in quicker juvenile development rates (Miller and Emlet, 1999; Phillips, 2002), increased survivorship (Emlet, 1986; Emlet and Hoegh-Guldberg, 1997), increased intra- and inter-specific competitive ability (Connell, 1985), and a size refuge from predation (Gosselin, 1997). Interestingly, juvenile size in echinoids shows no predictive correlation with egg size suggesting that juvenile size is dependent on larval experience only (Levitan, 2000).

Planktotrophic larvae by definition develop from eggs with only a portion of the energy necessary to reach metamorphic competence. The amount of maternal investment per egg determines when during development feeding becomes obligatory. The "onset of feeding" consists of two dissociable events: onset of the ability to feed and onset of the need for food to continue development (Herrera et al., 1996; Fig. 1). The interval of time between the onset of the ability and the onset of the need to feed is termed the "facultative feeding period." Similarly, the period of time from the onset of the need for food to metamorphosis is defined as the "obligate feeding period" (Herrera et al., 1996).

"Facultative feeding periods" are distinct from "facultative planktotrophic larvae" (Emlet, 1986; Hart, 1996; McEdward, 1997; Fig. 1). A facultative planktotrophic larva represents one extreme of facultative feeding periods where the offspring are provisioned with adequate energy within the egg to complete metamorphose without exogenous food. However, the larva retains the complex morphology typical of planktotrophic larvae with obligate feeding periods despite having adequate endogenous reserves to metamorphose. Species with facultative planktotrophic larvae have been reported for echinoids (Emlet, 1986; Hart, 1996), nudibranchs (Kempf and Hadfield, 1985; Kempf and Todd, 1989) and an amphibian (Crump, 1989). At the other extreme of facultative feeding periods in larval development are species that require food directly after differentiation of the mouth and digestive system to continue development. This type of feeding strategy may result from a minimal maternal energetic investment or from a dependence on an exogenous factor (e.g. mineral, hormone) for further development. An example of this nutritional type may be represented by some crustaceans with early feeding requirements to reach successive molt stages (Anger, 1987). Therefore, all planktotrophic larvae have some period of facultative feeding during the course of larval development that will usually be determined by the quantity of maternal investment.

A number of authors have studied the continuum of feeding nutritional strategies in planktotrophic

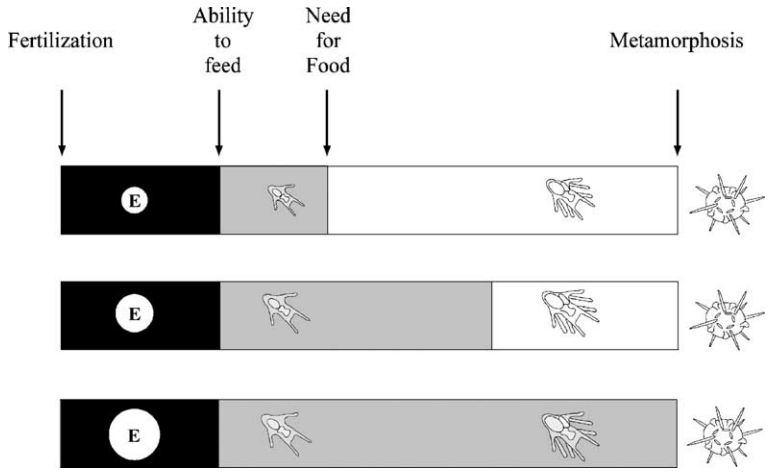

Fig. 1. What is the facultative feeding period in planktotrophic larvae? The bars from top to bottom represent life history to metamorphosis of planktotrophic larvae that develop from eggs (E) to metamorphosis with increasing amounts of maternal investment (egg energy). Three periods can be distinguished. Black=pre-feeding period; Grey=facultative feeding period; White=obligate feeding period. The duration of the pre-feeding period is reported to be independent of egg size for echinoids (Dickie et al., 1989). The bottom bar represents a facultative planktotrophic larva where the larva retains the ability to feed despite having sufficient energy to reach metamorphosis without food. Note that the length of the planktonic period (fertilization to metamorphosis) can be different for species developing from different sized eggs and has been normalized here.

echinoid larvae (e.g. Strathmann, 1985; Emlet, 1986; Eckert, 1995; Hart, 1996; Herrera et al., 1996). Whereas all feeding echinoid larvae are capable of feeding by the same early developmental stage (4-arm pluteus), great differences exist in the larval stage at which feeding becomes necessary for continued development and growth (Emlet, 1986; Eckert, 1995; Herrera et al., 1996). The degree of maternal investment is positively correlated with the stage when feeding becomes obligatory (Miner et al., unpublished data). Larvae developing from a large egg have a longer facultative period (measured in percent of total developmental time, not necessarily number of days) than larvae developing from a more poorly provisioned egg.

In this study, we quantified the influence of feeding versus not feeding during the facultative feeding period on two important characters in echinoid life histories: larval development time and energy at metamorphosis. With a series of experiments we approached three questions, quantifying the effects on these two characters: What is the effect of feeding during the facultative feeding period in obligate planktotrophic echinoid larvae? Does the concentration of food in the environment influence the effect of facultative feeding periods? Does the degree of maternal investment influence the relative impact of feeding in the facultative period?

We present research evaluating the contribution of the facultative feeding period to larval development and energy at metamorphosis in two closely related clypeasteroids, *Mellita tenuis* and *Leodia sexiesperforata*, differing in egg size and organic content. We used closely related species with similar geographic distribution to minimize confounding factors such as adaptations to different ecological conditions (e.g. temperature, salinity) (Pechenik, 1999) or clade-specific larval forms (Wray, 1992, 1995). Experiments for each species were conducted at food limiting (food concentration below amount necessary for maximal development rate) and nonlimiting conditions (food concentration necessary for maximal developmental rate) to assess the impact that food environment may have as well. Our results indicate that both egg investment and food environment differentially affect how the facultative feeding period contributes to echinoid development for these two species. We then use our results to make comparisons with outcomes from McEdward's model (1997) to evaluate if the experimental data and theoretical predictions are consistent.

2. Material and methods

2.1. Adult collection and larval culturing

We collected adult *M. tenuis* west of Cedar Key, Florida in May 2001 and July 2004 by SCUBA. Adult *L. sexiesperforata* were collected by snorkeling off Long Key, FL in June 2001 and May 2004. Animals were maintained in aquaria with recirculating seawater. Spawning was induced with an intracoelomic injection of 0.55 M KCl (Strathmann, 1987). Eggs were collected from a single female for each species at each collection date (2001 and 2004) in separate 100 ml glass beakers containing 0.45-μm-millipore-filtered-seawater (MPFSW). Sperm was collected "dry" from a single male for each species after injecting with KCl. For each species we rinsed the eggs three times with MPFSW and then fertilized (>98% fertilization). Egg diameters of 20 fertilized eggs were measured for each species using an ocular micrometer on a compound microscope.

Larval cultures were maintained in 2 L glass beakers at concentrations of 1 individual per 4 ml MPFSW. Cultures were maintained in an incubator at 28 °C and water in each larval culture was changed every 2 days. Larvae in cultures were fed after each water change at levels determined by the treatment to which they had been assigned. The position of cultures within the incubator was randomized each time the water was changed. Algae (*Dunaliella tertiolecta* Butcher) were cultured in f/2 enriched seawater nutrient medium (Guillard, 1975; Strathmann, 1987) and were washed by centrifugation and resuspension in seawater.

2.2. Experimental design

Larvae from each of the two species, *L. sexiesperforata* and *M. tenuis* were placed in one of four food treatments: (0–2)=no food during facultative period, 2 cells/μl *D. tertiolecta* during obligate period; (2–2)=2 cells/μl *D. tertiolecta* during both the facultative and the obligate period; (0–6)=no food during facultative period, 6 cells/μl *D. tertiolecta* during obligate period; (6–6)=6 cells/μl *D. tertiolecta* during both the facultative and the obligate period (for explanations on feeding periods see Fig. 1). Length of the facultative period for both *M. tenuis* and *L. sexiesperforata* had been previously determined to be 2.5 days post-fertilization at 28 °C (Miner et al., unpublished data). We considered 6 cells μl^{-1} of *D. tertiolecta* as nonlimiting food condition and 2 cells μl^{-1} of *D. tertiolecta* as limiting food condition (see Herrera, 1998).

2.3. Time to metamorphosis

Metamorphic competence in the larvae was determined through daily assays of metamorphic competence for each replicate beaker of each treatment for each species. We completed a more rigorous and thorough determination of time to metamorphic competence with individuals from the 2004 cultures in order to determine how metamorphic competence varies over time. Start date for metamorphic assays was determined through visual inspection of larvae such that when juvenile rudiments were observed in the feeding treatment, individuals of all replicates of that treatment were assayed. Larvae were induced to metamorphosis by exposure to 40 mM excess K^+ (Cameron et al., 1989). One subset of 10 larvae was removed from each replicate culture and incubated in the K^+-enriched seawater for up to 1 h to check for metamorphosis. For the 2001 analyses, when an average of more than 50% of individuals metamorphosed within these subsets, we then exposed the remainder of the cultures to the K^+-enriched seawater for biochemical analysis. Juveniles were collected and placed in fresh filtered seawater for biochemical analyses.

2.4. Biochemistry

Biochemical analysis (protein, lipid and carbohydrate) was performed on newly spawned, fertilized eggs and newly metamorphosed juveniles from 2001. Five replicate samples per species per treatment (see Table 1 and Appendix A for number of individuals used per replicate) were prepared in micro-grinders (100–1000 μl capacity, Fisher Scientific) and seawater was removed with a micropipette.

Protein was quantified with the Coomassie brilliant blue G-250 binding assay (Bradford, 1976) with Bovine Serum Albumin (BSA) as the standard (0.0–7.5 μg carbon). Samples were homogenized in 700 μl distilled water and 600 μl aliquots were removed from

Table 1
Echinoid species with larger eggs have more energy in two of three biochemical classes

	Mellita tenuis	*Leodia sexiesperforata*	ANOVA
Lipids (n_{Ls}=40, n_{Mt}=30)	0.21±0.0037	0.51±0.015	($F_{1,8}$=54.86; p<0.001)
Carbohydrates (n_{Ls}=40, n_{Mt}=30)	0.078±0.025	0.10±0.0070	($F_{1,8}$=0.897; p=0.371)
Proteins (n_{Ls}=40, n_{Mt}=30)	0.049±0.0061	0.17±0.016	($F_{1,8}$=47.51; p<0.001)

L. sexiesperforata has a significantly higher lipid and protein content in eggs when compared with *M. tenuis*. Values represent µg±1 S.E. mean for five replicate samples per species collected in 2001 per biochemical type assayed. n_{Ls} indicated the number of eggs used for biochemical analysis in *L. sexiesperforata* and n_{Mt} indicated the number of eggs used for the biochemical analysis in *M. tenuis*.

each replicate and placed in 13×100 mm glass test tubes. Fifty microliters of Bio-Rad (Bio-Rad Protein Assay, Bio-Rad Laboratories, Hercules, CA) reagent was added to each tube and contents were mixed thoroughly. Samples were measured spectrophotometrically (595 nm, 1 cm path length). Bio-Rad's protein assay is based on the color change of Coomassie brilliant blue G-250 dye in response to various concentrations of protein. Protein content was calculated as the weight of BSA (µg) yielding equivalent color change. Total protein content for the original sample was calculated by correcting for the difference in volume between the 600 µl aliquot and the full volume of distilled water used in the extraction.

Lipid content was measured using the acid-dichromate oxidation technique (Parsons et al., 1984) with tripalmitin as the standard (0.0–50.0 µg carbon). Samples were homogenized in 200 µl chloroform and methanol at a 2:1 (v/v) ratio. Next, 50 µl of distilled water was added to each grinder and lipids were extracted by additional agitation and grinding with the pestle (Bligh and Dyer, 1959; Christie, 1982). Phases were allowed to separate and 100 µl aliquots of the organic phase were removed from each sample, placed in 13×100 mm acid-washed (0.3% acid dichromate) test tubes, and dried using a dry bath incubator at 65 °C for 2 h. Lipids were oxidized with potassium dichromate (0.30%) in concentrated sulfuric acid (400 µl, 15 min, 105 °C). Samples were diluted (900 µl distilled water) and measured spectrophotometrically (440 nm, 1 cm path length). Lipid content was calculated as the weight of tripalmitin (µg) yielding equivalent reduction in dichromate oxidation. Total lipid content for the original sample was calculated by correcting for the difference in volume between the 100 µl aliquot and the full volume of chloroform used in the extraction.

Carbohydrate content was assayed using the phenol-sulfuric acid method (Dubois et al., 1956) with dextran as the standard (0.0–20.0 µg carbon). Samples were homogenized in 150 µl of distilled water. One hundred microliter aliquots of each sample were placed in 1.5 ml plastic centrifuge tubes. One hundred microliters of liquefied phenol (Fisher Scientific) were added to each tube, followed immediately by 500 µl of concentrated sulfuric acid (H_2SO_4). Tubes were capped, mixed, and left at room temperature for 10 min. All samples were heated in a dry bath incubator at 30 °C for 20 min and mixed thoroughly. Samples were measured spectrophotometrically (490 nm, 1 cm path length). The phenol-sulfuric acid method is a colorimetric test that quantifies production of a yellow-orange product from reducing sugars and polysaccharides. Carbohydrate content was calculated as the weight of dextran (µg) yielding equivalent color change. Total carbohydrate content for the original sample was calculated by correcting for the difference in volume between the 100 µl aliquot and the full volume of distilled water used in the extraction.

Total energy (expressed in joules) was calculated by converting the data values from micrograms to joules (after McEdward and Morgan, 2001). The values for each biochemical class (proteins, lipids, carbohydrates) were summed to yield the total energy. Total energy calculated from summing individual components should be viewed with caution as our three assays will exclude some components of the individuals and cannot account for variation between individuals between assays within treatments (see Jaeckle, 1995; McEdward and Morgan, 2001).

2.5. Experimental analysis and statistics

Biochemical composition and diameter of eggs between *L. sexiesperforata* and *M. tenuis* was compared using one-way ANOVA. Similarly, time to metamorphosis was compared with two factor

ANOVA (food concentration, fed or unfed during facultative feeding period) between the four feeding treatments within a species. As described above, time to metamorphosis is defined as the day that the mean of the four replicate cultures within a feeding treatment exceeded 50%. We used this date as the time to test for juvenile energy composition between treatments. Due to variation in time to 50% metamorphosis between replicates within a treatment, we also statistically compared the date when each replicate from a particular treatment reached a minimum of 50% metamorphosis. We calculated energy uptake (ΔE) as the energy difference between juveniles (E_j) and eggs (E_e) and performed ANOVA for comparison of biochemical composition between food treatments within the same species. Data for all biochemical compounds were tested for normal distribution using the P–P plot command application in SPSS™. All statistics were performed using SPSS™.

3. Results

The mean egg diameter from the 2001 females for *M. tenuis* was 98.9±1.9 μm (N=20) and for *L. sexiesperforata* was 161.9+3.1 μm (N=20). For the 2004 females, the egg diameter for *M. tenuis* was 122.0±3.5 μm and for *L. sexiesperforata* was 201.2±4.7 μm, both significantly larger than the females used in 2001 (*M. tenuis*: $F_{1,39}$=550.31, p<0.001, *L. sexiesperforata*: $F_{1,39}$=965.38, p<0.001). Overall energy content of eggs in terms of μg lipids and μg proteins was significantly larger in *L. sexiesperforata* then in *M. tenuis* (Table 1). No significant difference in carbohydrate content was found between the two species. Egg energy was assayed only for batches of eggs from 2001 precluding a comparison with the eggs collected in 2004.

3.1. Time to metamorphosis

Time to metamorphosis for each species was in part dependent on degree of maternal investment and the specific food treatment. Figs. 2 and 3 report the time to metamorphosis from the 2004 individuals for all treatments from both species. For *L. sexiesperforata*, time to metamorphosis was assayed over 4 days from 4 to 8 days post-fertilization (Fig. 2). On day 4, only a small percentage of larvae metamorphosed in the 6–6 and 2–2 treatment with no metamorphosis in the other two treatments. Percentage of larvae that metamorphosed in each treatment increased in the subsequent days (Fig. 2A). Our cutoff value for determination of "time to metamorphosis" was determined when the treatment exceeded 50% metamor-

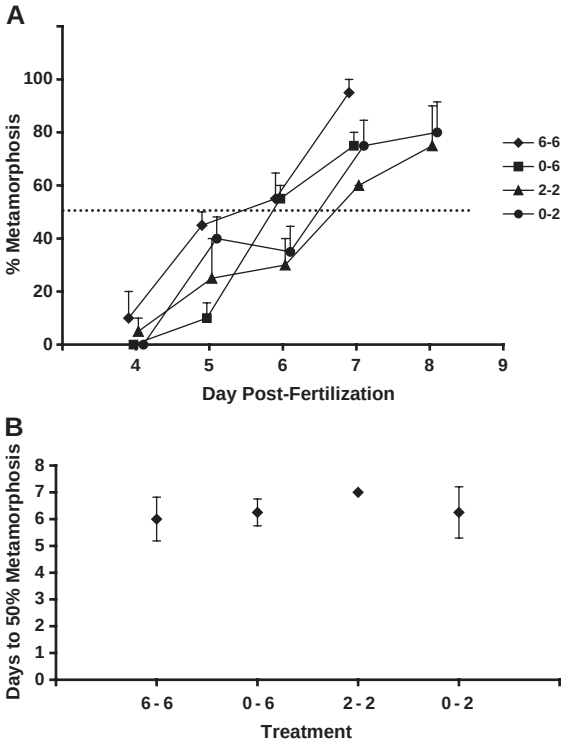

Fig. 2. The effect of two different food concentrations and feeding during the facultative feeding period on time to metamorphosis in *Leodia sexiesperforata*. While high food (6–6 or 0–6) accelerated development to metamorphosis in the large egg size species *L. sexiesperforata* compared to low food (2–2 or 0–2) when assessing what date the average percent metamorphosis was greater than 50% for all replicates in a treatment (A), there is no significant effect of feeding during the facultative period (0–6 compared to 6–6 and 0–2 compared to 2–2 respectively) nor feeding concentration on date when 50% metamorphosis was reached for each replicate within a treatment (B). Data points indicate mean±1 S.E. for four replicate cultures for each treatment. Figure legend abbreviations represent the four feeding treatment: (6–6)=6 cells/μl *Dunaliella tertiolecta* during both the facultative and the obligate period; (0–6)=no food during facultative period, 6 cells/μl *D. tertiolecta* during obligate period; (2–2)=2 cells/μl *D. tertiolecta* during both the facultative and the obligate period; (0–2)=no food during facultative period, 2 cells/μl *D. tertiolecta* during obligate period. The dotted horizontal line in A indicates 50% metamorphosis.

phosis (see ""Material and methods""). By this determination, larvae in both nonlimiting food ration treatments (0–6 and 6–6) had the same time to metamorphosis (6 days). Similarly, larvae in the two limiting food ration treatments (0–2 and 2–2) did not diverge in time to metamorphosis, both exceeding 50% metamorphosis on day 7. Time to metamorphosis was not significantly different between the limiting and nonlimiting food treatments when we measured date when each replicate beaker reached 50% metamorphosis ($F_{1,12}$=2.18, p=0.165) nor was there a significant effect of feeding during the facultative feeding period for either food concentration ($F_{1,12}$=0.55, p=0.474; interaction p=0.165). Thus, for *L. sexiesperforata*, time to metamorphosis was not significantly different for either food concentration in the culture during the obligate feeding period or the presence of food during the facultative period despite the trend for on average shorter development time in higher food concentration.

For *M. tenuis*, time to metamorphosis was assayed over 12 days from 8 to 20 days post-fertilization (Fig. 3). On day 8, only a small percentage of larvae metamorphosed in the 6–6 treatment with no metamorphosis in any other treatment. In general, a higher concentration of food resulted in shorter times to metamorphic competence regardless if larvae were fed during the facultative feeding period or not (Fig. 3A). There was an overall significant difference in time to 50% metamorphosis between treatments ($F_{1,12}$= 69.87, p<0.001) as well as between fed and unfed during the facultative feeding period ($F_{1,12}$=6.97, p=0.022, interaction p=0.02, Fig. 3B). Larvae in nonlimiting food ration treatments (0–6 and 6–6) significantly differed in time to metamorphosis. Larvae in the 6–6 treatment reached metamorphic competence 10 days post-fertilization and larvae in 0–6 treatment metamorphosed in 13 days ($F_{1,7}$=19.64, p=0.004) indicating that feeding during the facultative feeding period influenced time to metamorphosis. In contrast, larvae reared in limiting food conditions (0–2 and 2–2) did not significantly differ in time to metamorphosis with most treatments reaching metamorphic competence on day 16 ($F_{1,7}$=0.7714, p=0.4136). Thus, for *M. tenuis*, time to metamorphosis was dependent on level of food concentration in the culture during the obligate feeding period, but presence of food during the facultative feeding period

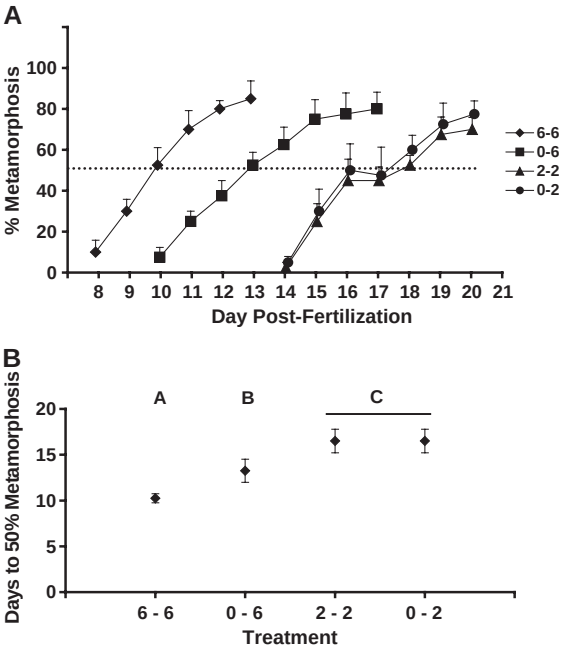

Fig. 3. The effect of two different food concentrations and feeding during the facultative period on time to metamorphosis in *Mellita tenuis*. Both higher food concentration and feeding during the facultative period accelerates time to metamorphosis. Note that the effect of feeding during the facultative period is only significant under high food conditions (6–6 vs. 0–6). See Fig. 2 for description of legend.

significantly affected development time only at nonlimiting food concentration.

For the larvae we assayed in 2001, times to metamorphosis showed a qualitatively identical pattern as the 2004 individuals. *L. sexiesperforata* metamorphosed in 60% of the time that *M. tenuis* needed to develop to metamorphosis under constant nonlimiting food conditions (6–6) and in 47% of the time under limiting food conditions (2–2). Time to metamorphosis for *M. tenuis* was prolonged when larvae were unfed during the facultative period and fed a nonlimiting food ration during the obligate feeding period (0–6 versus 6–6). At a limiting food ration (0–2 versus 2–2), however, we found no effect of fed versus unfed during the facultative period on time to metamorphosis in either *M. tenuis*. Larval development of *L. sexiesperforata* was prolonged by approximately one to two days under limiting food conditions (0–2 and 2–2) versus nonlimiting food conditions (0–6 and 6–6). There was, however, no

effect of feeding during the facultative period within limiting or nonlimiting food treatments on the time to metamorphosis for *L. sexiesperforata*.

3.2. Biochemistry

We report a significant increase of lipids and carbohydrates in *M. tenuis* when fed during the facultative period at a nonlimiting food ration (6–6) compared to larvae that were starved during the facultative period (0–6, Table 2; Appendix A). For mean values and standard errors of the mean of all energy types for both species in μg and joule refer to Appendix A. No significant difference in juvenile proteins, lipids, or carbohydrates was found at the limiting food ration for *L. sexiesperforata* (2–2, 0–2). The only significant effect of feeding during the facultative period on juvenile energy in *L. sexiesperforata* was a small decrease in protein content for those fed during the facultative period at nonlimiting food concentration (Table 2; Appendix A).

We furthermore compared ΔE from *L. sexiesperforata* with ΔE from *M. tenuis* for the treatments 2–2 and 6–6 to test the hypothesis that a species with a relatively long facultative period (*L. sexiesperforata*) accumulates more energy during development to metamorphosis compared to a species with a relatively short facultative period (*M. tenuis*). Since this experiment was not replicated, we were not able to statistically compare energy accumulation between species. By comparing the mean total energy between species in the same food treatment, there is a two-fold difference between the mean energy accumulated during the facultative period by *L. sexiesperforata* and *M. tenuis* at nonlimiting food (0.0606 J and 0.121 J respectively) and a one and one-half fold difference in limiting food (0.0523 J and 0.0810 J respectively). When standardized for total time spent in the larval period (i.e. days to metamorphosis), these differences in metamorph energy between species are largely negated. The mean values and standard errors of the mean for all energy types are listed in Appendix A.

4. Discussion

The impact of feeding during the facultative period on larval development and juvenile energy for two echinoid species varied with quantity of maternal investment and food concentration. There was no broad advantage to facultative feeding for both of these species either to decrease time to metamorphosis or to increase the mass of all biochemical components in the juvenile stage.

Maternal investment influenced the impacts of facultative feeding on larval development and metamorph energy. Under nonlimiting food conditions in the small egg planktotroph *M. tenuis*, feeding during the facultative period resulted in a shorter development time and juveniles with more energy (lipids and carbohydrates) when compared with siblings unfed during the facultative period. This result corresponds with our prediction since the fed larvae spent more time overall feeding and would be expected to have

Table 2
Influence of food ration during the facultative period on juvenile biochemical composition of *Mellita tenuis* and *Leodia sexiesperforata* collected in 2001

		Lipids	Carbohydrates	Proteins
L. sexiesperforata	Limiting food ration	0.50±0.51	−0.092±0.060	0.015±0.070
	(2–2, 0–2)	(p=0.34; n=5)	(p=0.13; n=5)	(p=0.85; n=5)
	Nonlimiting food ration	0.94±0.51	0.044±0.060	−0.16±0.070*
	(6–6, 0–6)	(p=0.08; n=5)	(p=0.45; n=5)	(p=0.05; n=5)
M. tenuis	Limiting food ration	−0.073±0.28	0.078±0.15	0.018±0.060
	(2–2, 0–2)	(p=0.80; n=5)	(p=0.62; n=5)	(p=0.77; n=5)
	Nonlimiting food ration	0.80±0.28*	0.66±0.15*	−0.099±0.060
	(6–6, 0–6)	(p=0.01; n=5)	(p<0.01; n=5)	(p=0.13; n=5)

Values indicate mean differences±1 S.E. in content (μg) between juveniles that were starved and fed during the larval facultative feeding period. Positive values indicate higher values when fed during the facultative period. These differences were compared with ANOVA. Note that comparisons are based on marginal estimated means. n represent the sample sizes of starved and fed treatments that the analysis was based on. Significant differences (p<0.05) are indicated by an *.

quicker development and larger nutritional reserves. By feeding during the facultative period, an individual larva can decrease development time and thereby reduce pelagic mortality risks. Conversely, larvae of *L. sexiesperforata* (the large egg planktotrophic species) that were fed in the nonlimiting food treatment during the facultative period had neither a significantly shorter time to metamorphosis nor larger juvenile energy when compared with those that were starved during this period of development. In fact, *L. sexiesperforata* juveniles fed during the facultative period at nonlimiting food concentrations had significantly lower amounts of protein compared with those larvae that were unfed during this same period. With the present data we cannot determine why the amount of protein decreased, but this significant decrease could be due to the induction of digestive enzymes or limited protein synthesis relative to degradation.

Secondly, the effect of feeding during the facultative period depended on food concentration. In the limiting food experiments, facultative feeding did not reduce development time to metamorphosis nor were juveniles more energy rich for either species. By our two measures of evaluating the influence of the facultative period (i.e. development time and juvenile composition), the benefits of facultative feeding are absent. Thus the benefits of facultative feeding were decreased in lower food environments indicating that if the food environment was of relatively poor concentration or quality, larvae could feed or not feed during this period with no measurable effect on development.

McEdward (1997) evaluated the importance of the facultative feeding period for planktotrophic larvae with a fecundity–time model. Maximal reproductive success could be achieved at intermediate levels of investment per offspring dependent on level of food available in the environment. This expectation contrasts with Vance's (1973) time–fecundity model that predicts extreme investment strategies to be the only evolutionarily favorable strategies. Vance's model did not allow for feeding during the facultative feeding period. The difference in output for the McEdward (1997) model was in allowing feeding during this period. In high food environments, larvae developing from poorly provisioned eggs could accumulate a portion of the energy needed to metamorphose during the facultative period thereby reducing total pelagic time. A reduction in pelagic time increases the portion of offspring surviving and thus fitness. Conversely, in low food environments, larvae should gain less benefit from feeding during the facultative feeding period. In these cases, the McEdward model predicts that only the extreme minimum and maximum investment strategies would be evolutionary favored, similar to models such as Vance (1973).

Our data show that the benefits of facultative feeding periods in planktotrophic larvae are partially consistent with the theoretical expectations proposed by McEdward (1997). Despite only having data from two species, we can make an initial evaluation of the model's assumptions and the likely impact on the model. In the small egg planktotroph *M. tenuis*, feeding during the facultative period under nonlimiting food conditions resulted in a shorter development time. Facultative feeding benefits a larva developing with little egg provisioning by providing a period of time to ingest exogenous food while still developing from maternal energy invested in the egg. The energy from the ingested food is then available for continued development when the egg energy is depleted. Interestingly, we did not see the same effect of feeding during the facultative period for *L. sexiesperforata*, the species with more egg provisioning. This result is inconsistent with the McEdward (1997) model where feeding during the facultative period should have reduced development time. We suggest that this result may be due to reduced food assimilation or feeding efficiency in this species, a factor not incorporated into the McEdward (1997) model. Hart (1996) found that the facultative feeding larva of *Brisaster latifrons* had reduced feeding efficiency when compared to an obligate planktotrophic species. Similar results were found in another facultative feeding echinoid larva, *Clypeaster rosaceus* (Reitzel and Miner, unpublished data). This may suggest that species with a decreased dependence on exogenous food may develop less efficient feeding and digestive machinery. Additional energetic data with well-provisioned planktotrophic larvae will add needed information to address this question.

Our result showing no significant effect of the facultative feeding period in low food environments for either species is consistent with the McEdward (1997) model. When food concentration is low, the quantity of food ingested in a facultative feeding

period is likewise low. As predicted by the model, we would not expect feeding during the facultative feeding period in a low food concentration to have a significant effect on larval development.

The lack of a clear reduction in development time with facultative feeding in *L. sexiesperforata* is consistent with results reported for facultative planktotrophic larvae of the echinoid *Clypeaster rosaceus* (Emlet, 1986) as well as other invertebrates (Hentschel and Emlet, 2000 and references therein). When an adult is producing energetically rich enough eggs to produce facultative planktotrophic larvae, the larvae develop at the maximum rate to metamorphosis without exogenous food. Facultative feeding periods in well-provisioned obligate planktotrophs can allow this same developmental result by allowing exogenous food accumulation during the facultative feeding portion of development to provide the rest of the necessary energy to reach metamorphosis (unpublished lab results with *L. sexiesperforata*). Therefore, the effect of maternal investment and feeding during facultative feeding periods is similar to "determinate metamorphic ages" after a certain period of larval development reported by Hentschel and Emlet (2000). Well-provisioned offspring develop to a late stage before becoming dependent on exogenous food and thus may commit to metamorphosis, thereby limiting the plasticity in age at metamorphosis. Further work with species with well-provisioned planktotrophic larvae could further support this hypothesis.

The results for *M. tenuis* provide information not contained in fecundity–time models, as juvenile energy was significantly different in addition to development time. Hentschel and Emlet (2000) and Phillips (2002) reported similar results for nauplii and veligers, respectively, where individuals fed under variable food concentrations differed in time to metamorphosis and metamorph size. The exclusive emphasis on development time in fecundity–time models for maternal investment strategies may be difficult to justify in light of these results. Juvenile energy has not been quantitatively modeled to assess potential influences on life history evolution for marine invertebrates but has been qualitatively considered multiple times (e.g. Strathmann, 1977; Eckert, 1995; Hentschel, 1999; Hentschel and Emlet, 2000; Phillips, 2002).

Higher juvenile energy due to larval experience may have a significant influence on the evolution of life histories. Increased juvenile energy may be important to decrease the probability of benthic mortality (Perron, 1986; Emlet and Hoegh-Guldberg, 1997; Phillips, 2002). Heavy mortality in the larval phase is frequently cited as driving marine invertebrate reproductive strategies and has thus received much attention (Rumrill, 1990). However several studies suggest that benthic selection pressure on early juveniles may be equally as high resulting in extensive juvenile mortality for a cohort (Rowley, 1989; reviewed by Gosselin and Qian, 1997). Juvenile performance due to individual differences may have a significant influence on individual predation and competition (e.g. Emlet and Hoegh-Guldberg, 1997; Pechenik et al., 1998; Moran, 1999; Phillips, 2002). All other things being equal, more energy rich juveniles perform better than conspecifics of poorer quality. Having greater reserves may then allow for increased resistance to starvation or prolonged low food conditions, a shorter time to larger size to avoid size specific predation, and better competitive ability.

Further analysis detailing maternal investment and development strategy will demonstrate the diversity of feeding strategies present in marine invertebrates and how evolutionary transitions in developmental mode may occur. Other models, specifically Levitan (1993, 2000), have derived similar results to the McEdward model (i.e. evolutionary favored intermediate maternal investment strategies) but by considering other components of the life history (fertilization, development time). The relative impact of each component of the life history on the organism's complete life history warrants further attention both theoretically and empirically. We, like others (see above), suggest the inclusion of metamorph quality in future quantitative models for the evolution of organisms with indirect development.

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Appendix A

Average biochemical composition of *Leodia sexiesperforata* and *Mellita tenuis* after completion of metamorphosis. *N* indicates the number of juveniles per replicate biochemical analysis. All treatments had five replicates per treatment. See Fig. 2 for treatment descriptions.

	Treatment	Juvenile energy in μg	Juvenile energy in joule
L. sexiesperforata			
Lipid	0–2 (*n*=5)	1.26±0.19	0.050±0.0077
	2–2 (*n*=5)	1.76±0.32	0.070±0.013
	0–6 (*n*=2)	0.89±0.18	0.035±0.0070
	6–6 (*n*=5)	1.83±0.59	0.073±0.023
Carbohydrate	0–2 (*n*=8)	0.26±0.042	0.0046±0.00074
	2–2 (*n*=8)	0.17±0.029	0.0030±0.00051
	0–6 (*n*=5)	0.17±0.057	0.0029±0.0010
	6–6 (*n*=5)	0.21±0.025	0.0037±0.00044
Protein	0–2 (*n*=8)	0.21±0.054	0.0050±0.0013
	2–2 (*n*=8)	0.22±0.048	0.0053±0.0012
	0–6 (*n*=5)	0.57±0.045	0.014±0.0011
	6–6 (*n*=5)	0.41±0.062	0.0099±0.0015
M. tenuis			
Lipid	0–2 (*n*=5)	2.00±0.16	0.079±0.0064
	2–2 (*n*=5)	1.93±0.17	0.076±0.0068
	0–6 (*n*=2)	1.91±0.16	0.076±0.0065
	6–6 (*n*=2)	2.72±0.28	0.11±0.011
Carbohydrate	0–2 (*n*=5)	0.13±0.053	0.0024±0.00093
	2–2 (*n*=8)	0.21±0.091	0.0037±0.0016
	0–6 (*n*=5)	0.16±0.088	0.0028±0.0015
	6–6 (*n*=5)	0.82±0.17	0.014±0.0029
Protein	0–2 (*n*=5)	0.46±0.042	0.011±0.0010
	2–2 (*n*=8)	0.48±0.045	0.012±0.0011
	0–6 (*n*=5)	0.42±0.052	0.010±0.0013
	6–6 (*n*=5)	0.32±0.032	0.0078±0.00076

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