Growth, development and condition of *Dendraster excentricus* (Eschscholtz) larvae reared on natural and laboratory diets

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Feeding invertebrate larvae may be food limited while developing in the ocean. If they are, then their time in the plankton is prolonged, which likely increases mortality. Food limitation could be due to the quantity and/or quality of the food available. In an effort to answer how food type influences larval nutrition, we compared growth, development and lipid deposition for *Dendraster excentricus* larvae reared in natural seawater from two depths (1 and 20 m) and in filtered seawater on a monoculture laboratory diet of 6 cells µL⁻¹ of the green alga *Dunaliella tertiolecta* (Butcher). Five days post-fertilization, larvae reared on the laboratory diet had developed to the latest stage, were the largest and had lipid deposits. Larvae reared on natural surface water were intermediate in size and developmental stage, and larvae reared in the water from 20 m depth were the smallest and developed the slowest. This trend continued at 8 days post-fertilization when surface water diet larvae were similar in size to laboratory diet larvae, but their juvenile rudiments were significantly smaller. To assess food availability in each food treatment, we compared the concentration of chlorophyll (Chl) a, b and c in natural seawater from each depth and in *D. tertiolecta* culture in filtered seawater. Natural seawater collected from the surface had the highest concentration of Chl a and c, whereas Chl b was not significantly different between treatments. Increased Chl concentrations in the surface water are likely due to higher concentrations of diatoms and dinoflagellates, which are typically not high-quality food items for echinoid larvae. Our results support a hypothesis that echinoid larvae in the water column may be limited by food quality.

INTRODUCTION

Previous studies have suggested that the food concentration in the water column limits growth and development of marine invertebrate larvae (Conover, 1968; Olson and Olson, 1989). In food-limited conditions, development time may be prolonged, imposing increased mortality risks due to predation, offshore transport to unsuitable sites, or exposure to intolerable temperatures or salinities ([Lamare and Barker, 1999]; reviewed by [Rumrill, 1990]). Life history models suggest that development time can be a key parameter influencing the evolution of annual reproductive periods (Olive, 1992; Starr et al., 1993) and maternal investment strategies (Vance, 1973; McEdward, 1997; Levitan, 2000).

Studies investigating food limitation in the environment have principally been of two types. Correlative studies compare larval food supply with recruitment success [e.g., (Birkeland, 1982; Incze et al., 1987)]. Although informative for understanding relationships of natural plankton conditions and recruitment, these studies do not demonstrate the mechanism of decreased or elevated recruitment success and therefore cannot solely be used to identify food limitation. The second type of study designed to assess food limitation is laboratory nutrition studies in which larvae are reared on a limited variety of algae (typically a monoculture) to quantify the effect of diet on development time and growth rate (Stone, 1989; McEdward and Herrera, 1999; Miller and Emlet, 1999; Campbell et al., 2001). However, results from this type of experiment are difficult to extrapolate to the field where larvae are consuming a mixed diet of multiple algal species and potentially other micro- and nanoplankton (Rivkin et al., 1986; Moal et al., 1996), as well as absorbing dissolved organic matter (Manahan et al., 1983). Interpretation of
both types of studies is limited due to a lack of knowledge about the natural diet of larvae in the plankton. Quantifying larval development rates when reared under natural conditions is crucial for making realistic estimates of development time in the field.

There have been few studies investigating how larval growth and development relate to natural levels of plankton in the water column. Paulay et al. (Paulay et al., 1985), Fenaux et al. (Fenaux et al., 1994) and Eckert (Eckert, 1995) tested food limitation in the plankton by rearing larvae of several species in natural seawater. All three studies found that larvae developed faster when natural water was supplemented with additional algae, suggesting food limitation in the natural environment. Similarly, Olson (Olson, 1987) and Hansen (Hansen, 1999) tested how the development of an asteroid and three species of polychaete larvae, respectively, varied between natural plankton and an enriched plankton diet (addition of laboratory algae). In contrast to the studies of Paulay et al. (Paulay et al., 1985) and Fenaux et al. (Fenaux et al., 1994), Olson (Olson, 1987) and Hansen (Hansen, 1999) found that an enhanced diet did not significantly increase larval growth or settlement success.

These studies do not allow for a direct comparison of larval growth and development between individuals reared on natural food and larvae reared exclusively on a typical laboratory diet (algal monoculture). This comparison is a key link between empirical data from larval field studies and laboratory studies. In addition, studies involving natural food sources often collect samples from the surface where plankton are likely to be present in greater concentrations than in other portions of the water column. Vertical segregation of food is especially apparent in stratified bodies of water such as bays or temperate oceans with a summer thermocline (Cushing, 1989; Dekshenieks et al., 2001). Larvae may differ in their vertical position in the water column [e.g. Dendraster excentricus (Emlet, 1986; Pennington and Emlet, 1986); scallop (Gallager et al., 1996)] and thus the food concentrations encountered by the larvae will vary depending on vertical placement. The quantitative impact on growth and development of larval vertical position in a stratified water column is unknown.

In this study we approached three questions. (i) How does growth and development of larvae reared in a monoculture of laboratory-reared algae relate to growth and development of larvae reared in natural plankton collected from the surface (1 m) and 20 m? (ii) Is there a relationship between artificial diets and natural food diets to permit a correlation between studies relating food levels to development of larvae? (iii) Does chlorophyll concentration predict the larval nutritional environment? By measuring developmental stage, morphology and neutral lipid reserves, we comparatively assessed larval growth and development of the echinoid D. excentricus in three food treatments: natural seawater from 1 m depth, natural seawater from 20 m depth and a monoculture of the green alga (chlorophyte) Dunaliella tertiolecta (Butcher). These measures are relevant fitness measures as decreased development time is likely related to decreased larval mortality (Rumrill, 1990; Lamare and Barker, 1999) and increased energy reserves increase the probability of survival in the juvenile stage (Emlet, 1986; Phillips, 2002).

METHOD

Fertilization and larval rearing

Dendraster excentricus adults were collected from the East Sound, Orcas Island, Washington, in late spring 2002. Adults were held in aquaria with flow-through, sand-filtered seawater at Friday Harbor Laboratories. Spawning was induced in early August with a 1 mL intracoelomic injection of 0.55 M KCl. Sperm from one male and eggs pooled from three females were collected for larval cultures following procedures in Strathmann (Strathmann, 1967). Prior to fertilization, the eggs were rinsed three times with 0.45 µm Millipore filtered seawater to remove any contaminants from the mother. Eggs were fertilized with a dilute sperm suspension (>95% fertilization) and cultured through the late gastrula stage in stirred 4 L jars incubated in a flow-through sea table (ambient water temperature ~11°C).

Approximately 100 larvae were placed in each of four 1 L replicates for the three food treatments: natural surface water (1 m), natural deep water (20 m) or 0.45 µm Millipore filtered seawater supplemented with the green alga D. tertiolecta (6 cells µL⁻¹). Cultures of D. tertiolecta were reared in f/2 medium at room temperature (~21°C). Seawater for the natural diet treatments was collected from upper East Sound, Orcas Island, in the morning on the first day of the experiment (i.e. the day adults were spawned), then every other day using a 4 L Niskin collection bottle. East Sound is a protected body of water that is frequently thermally stratified in the upper 5 m of the water column (Dekshenieks et al., 2001). Temperature by depth data were taken on the day we spawned the adults and showed thermal stratification at the collection site (~2°C change in temperature was observed within the upper 5 m of the water column). The median water depth at the collection site near Rosario Point, East Sound, was 23 m.

Water for food treatments in the experimental cultures was changed by reverse filtration every 2 days. Surface and 20 m seawater treatments were collected on the mornings of initial set-up and culture changes. Before use in the larval cultures, all field-collected seawater was filtered through a 60 µm mesh in order to remove any competitive
zooplankton or algae too large for larvae to ingest (Strathmann, 1971). The abundance of phytoplankton was quantified by assessing chlorophyll (Chl) concentration (see below). Size and concentration of algal cells in the natural diet were not directly quantified. Cultures were maintained at room temperature (\(\sim 21^\circ C\)) and were mixed thoroughly 3–4 times daily by agitation with a glass rod. We did not see evidence of algal or larval settling in the cultures. Larval mortality in cultures, though not directly quantified, was judged to be low (<5%) for all treatments and replicates.

**Chlorophyll concentrations**

Chlorophyll \(a\), \(b\) and \(c\) concentrations for each treatment were measured as a proxy of phytoplankton abundance. The water samples used for Chl determination were subsamples of the water used for the rearing treatments. One liter of the prefiltered natural seawater from each depth was filtered through a 0.45 \(\mu\)m Millipore filter membrane. To quantify the Chl content of the \(D.\) tertiolecta fed to larvae, we filtered a volume of our stock algal culture containing the equivalent of \(6 \times 10^6\) cells \(L^-1\). Four replicates were filtered for each treatment. The filters were then placed in 10 mL of acetone in the dark for 24 h to extract the Chl. We recorded sample absorbance at wavelengths of 665, 645 and 630 nm using a Model 1601 Shimadzu spectrometer for four replicates of each treatment. We calculated the concentration of Chl (\(\mu\)g \(L^-1\)) according to equations from Strickland and Parsons (Strickland and Parsons, 1965).

**Larval growth, development and condition**

Differences in larval growth and development were analyzed by morphometric variation of larvae between replicates and treatments. The developmental stage of larvae was noted in each treatment and we measured postoral, posterodorsal and anterolateral arm lengths, as well as stomach length and width, to the nearest 0.01 mm [Figure 1; see (McEdward, 1986)]. When present, we also measured the lateral length of the juvenile rudiment. Measurements were taken from \(\times10\) and \(\times20\) digital microphotographs using image analysis software (NIH Image v. 1.62). Five larvae per replicate were haphazardly sampled and measured at two time points: 5 and 8 days post-fertilization. To assess the presence or absence of storage lipids (neutral lipids and waxy esters), five additional larvae from each replicate were stained with the lipophilic stain Nile Red (Hentschel, 1998; R. B. Emlet, personal communication). Nile Red is a hydrophobic fluorochrome that fluoresces yellow–orange in the presence of neutral lipids and red–orange when bound to polar lipids. Larvae were placed in a 1:4 dilution of stock Nile Red solution (2.5 mg of stain per 100 mL of acetone) to filtered seawater for 90 min and then rinsed in filtered seawater. Larvae were photographed from each replicate for lipids using a Nikon Model Y-FL fluorescent microscope at excitation wavelengths of 460–500 nm. The presence or absence of storage lipids was recorded for each larva.

**Statistical analysis**

We used nested ANOVAs followed by Tukey post-hoc analysis to evaluate differences for each measured larval character between treatments. We detected only one case of a significant effect of the replicate within a treatment. For the postoral arm length at the 5 day time point, there was a significant statistical effect between replicate beakers within the laboratory food treatment. This effect was not present in the second time point and had little effect on the relationships of the three treatments due to the large differences in the mean of the three treatments. For all other comparisons, there was no significant effect of the replicate on the analysis between treatments. We also performed ANOVAs followed by Tukey post-hoc analysis for statistical comparisons of the Chl \(a\), \(b\) and \(c\) content of each food treatment. All tests were conducted at the 5% significance level. The statistical package SPSS was used for both sets of analyses.

**RESULTS**

**Chlorophyll concentration**

Concentrations of Chl \(a\), \(b\) and \(c\) were highest in the surface water from East Sound (Figure 2). Chlorophyll \(a\) in surface
water (1.80 µg L⁻¹) was significantly higher than in the other two treatments (D. tertiolecta: 1.38 µg L⁻¹, 20 m water: 0.52 µg L⁻¹, F₂,₉ = 36.48, P < 0.0001). For Chl b, there was no significant difference between any of the treatments (surface: 0.753 µg L⁻¹, 20 m water: 0.359 µg L⁻¹, D. tertiolecta: 0.566 µg L⁻¹, F₂,₉ = 4.16, P = 0.0526) and all values were low compared with the other Chl types. Chlorophyll c in the surface water was significantly higher than in either the laboratory diet or the 20 m water (surface: 6.35 µg L⁻¹, 20 m water: 2.59 µg L⁻¹, D. tertiolecta: 2.24 µg L⁻¹, F₂,₉ = 10.51, P = 0.0044).

Larval growth, development and condition

Larval D. excentricus reared on the diet of D. tertiolecta had the greatest growth and fastest development rates of all the food treatments. Five days post-fertilization, 80% of sampled larvae reared on only D. tertiolecta were at the 8-arm stage with ectodermal invaginations indicating onset of growth of the juvenile rudiment. A majority (85%) of larvae reared in the surface water were at the early 8-arm stage without noticeable invaginations, and 90% of larvae in the 20 m water were at the early 6-arm stage. At 5 days post-fertilization, larvae reared on D. tertiolecta grew the most, followed by larvae reared on the surface water, then larvae reared on the 20 m water (Figure 3A). For each growth character (arm length, stomach length and body length), larval growths in all treatments were significantly different from one another (P < 0.05). In a qualitative assessment of lipid reserves, we observed lipid reserves in the stomach wall of only larvae reared on D. tertiolecta. In 90% of sampled larvae reared in this treatment, there were extensive lipid deposits throughout the entire stomach wall (Figure 4). We did not observe lipid deposits in any of the larvae reared on the natural food diets.

The second set of measurements (8 days post-fertilization) showed similar patterns in larval growth and development to those observed at 5 days post-fertilization (Figure 3B). Larvae fed only D. tertiolecta were at a similar stage (90% at 8-arm stage with rudiment) as the larvae cultured in surface water (75% at 8-arm stage with rudiment), but rudiment size of larvae fed D. tertiolecta was significantly larger than that of larvae reared on natural seawater (F₉,₅₇ = 59.67, P < 0.0001). Larvae reared in the 20 m water were less well developed (60% at early 8-arm stage without ectodermal invaginations, 25% at 6-arm stage) and were significantly smaller in body size (F₉,₅₇ = 30.61, P < 0.0001) and postero dorsal arm length (F₉,₅₇ = 16.59, P < 0.0001) than larvae reared in either the surface water or D. tertiolecta treatments.

DISCUSSION

In this study we quantified the effect of natural food and diets of cultured D. tertiolecta on larval growth and development of D. excentricus in order to provide a link between studies that examine only laboratory or field larval development. All measurements indicated a consistent relationship between development rate (i.e. larval stage), growth (i.e. body midline, arm length) and lipid stores, indicating that these measurements are representative of overall larval condition. Therefore, we conclude that laboratory-reared larvae fed a standard algal diet were less food limited than those reared on the water from the natural environment.

Our data agree with previous conclusions that echinoid larvae are food limited in the natural environment (Paulay et al., 1983; Fenaux et al., 1994). Food limitation is generally attributed to a low density of phytoplankton particles in the environment. Food limitation by availability of phytoplankton is likely responsible in our comparison of surface water with 20 m water where decreased larval growth and development correlated with low Chl content. The result is ecologically relevant as it provides empirical data for the effect of larval position in the water column on larval growth and development. This effect may be particularly relevant to species of zooplankton with diurnal migrations (e.g. D. excentricus larvae (Emlet, 1986; Pennington and Emlet, 1986); scallop larvae (Tremblay and Sinclair, 1990; Gallagher et al., 1996); annelid larvae (Thiebaut et al., 1992]). Field sampling of D. excentricus larvae by Emlet found early stage larvae in only intermediate to deep
water (6–15 m) in East Sound, whereas later stage larvae were largely concentrated at the surface (Emlet, 1986).

Food limitation may also be due to abundant phytoplankton that cannot be ingested or are of poor nutritional content (Hinegardner, 1969; Sterner and Hessen, 1994). We measured greater concentrations of all Chl types in the surface water than in either the laboratory diet or deep water. The concentration of phytoplankton is typically quantified by measuring the concentration of Chl a in water samples, despite some noted problems with accurate representation of the phytoplankton community ([Jasprica and Caric, 1996] and references therein). Our estimates of Chl a concentration agree with other published values for the San Juan Islands (Paulay et al., 1985). In our analysis, we have also included Chl b and c to obtain a more complete indication of the phytoplankton community at the time of sampling. As diets of planktonic larvae are largely unknown, we attempted to account for as much of the plankton community as possible. Our estimate of food concentration in natural seawater is a conservative

![Fig. 3. Mean + 1 SD sizes of larvae reared in the laboratory with *D. tertiolecta*, natural seawater from 1 m deep or natural seawater from 20 m deep at (A) 5 days post fertilization and (B) 8 days post fertilization. For 5 day larvae (A), all treatments are significantly different from one another (*P* < 0.05; *N* = 20) for each character. For 8 day larvae (B), treatments marked with an upper case letter are significantly different from those with a corresponding lower case letter (*P* < 0.05; *N* = 20). For a description of the abbreviations, see Figure 1.](https://academic.oup.com/plankt/article/26/8/901/1477768)
estimate of food availability, as many potential food items do not contain Chl (e.g. bacteria, heterotrophic protozoa). Despite the fact that the algal concentration (as measured by Chl concentrations) was highest in the surface water, larvae reared on the *D. tertiolecta* diet grew to be the largest, developed fastest and had more lipid deposits than larvae reared on either surface or bottom water. Similarly, Durbin *et al.* found food limitation in adult copepods (*Acartia tonsa*) despite relatively high Chl concentrations (Durbin *et al.*, 1983).

The concentration of Chl *c* was particularly high in the surface water, approximately three times higher than the other two treatments. Chlorophyll *c* is principally found in the ‘chromophyte’ algae, including diatoms and dinoflagellates (Dring, 1990). Studies have indicated that echinoid larvae (Hinegardner, 1969; Strathmann, 1987) develop poorly when fed a diet of various diatom species without supplementation of additional algal species. Obayashi and Tanoue suggested that zooplankton in general preferentially feed on algae containing Chl *a* and *b*, but not Chl *c* (Obayashi and Tanoue, 2002). If the majority of algae in the natural plankton samples were indeed diatoms, as suggested by the Chl *c* concentration and our visual inspections, then despite the high density of phytoplankton in the cultures, the average nutritional quality of these particles may not be as high as that provided by *D. tertiolecta* for these echinoid larvae.

We argue that growth and development of *D. excentricus* are subject to nutrient limitation and potentially sublethal toxicity due to unsuitable food quality, not inadequate food supply (as indicated by particle loading) in the upper portion of the water column. Sublethal toxicity of potential planktonic food sources for zooplankton, especially cyanobacteria and dinoflagellates, resulting in reduced growth or reproduction has been reported for a number of pelagic organisms (Demott and Moxter, 1991; Kirk and Gilbert, 1992; Bagoien *et al.*, 1996; Colin and Dam, 2003). Further analysis detailing phytoplankton species composition and abundance in the plankton coupled, with nutritional value to consumers, would provide further insight into the growth and development of larvae in the field. These investigations will be important not only for understanding the role of natural plankton composition in larval growth and survival, but also for successful aquaculture.

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