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More than morphology: Differences in food ration drive physiological plasticity in echinoid larvae

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ABSTRACT

Previous studies examining echinoid larval development have observed food-dependent allometric growth in morphological features related to particle capture ability. The aim of the current study was to determine if in conjunction with this morphological plasticity, there was also evidence for physiological plasticity (i.e., food-dependent differences in mass-specific rates and/or energetic efficiencies). Larvae of the Pacific sand dollar, *Dendraster excentricus*, were fed relatively low and high amounts of algae (1,000 and 10,000 algal cells ml^{-1}, respectively). Morphological plasticity was observed, with low-fed larvae possessing longer ciliated bands and postoral arms relative to midline body length and stomach length. Rates of ingestion, metabolism, and growth (protein and lipid biomass) were measured throughout larval development to investigate differences in larval energy partitioning and growth efficiencies. Mass-specific rates of ingestion and respiration were ~2-fold higher in high-fed larvae, indicative of significant differences in physiological state. While total energy ingested reflected the 10-fold difference in feeding concentration, partitioning of energy between growth and metabolism was significantly different. Low-fed larvae possessed higher ingestion-related efficiencies (assimilation, gross growth, and protein growth efficiencies) that decreased with age. High-fed larvae exhibited consistently greater values of net growth efficiency throughout development. This increase was driven by increasing metabolic efficiency of protein deposition. A correlative analysis between rates of ingestion and metabolism demonstrated that both low- and high-fed larvae had a similar cost of feeding of 0.29 (± 0.01; SE) μJ (algal cell ingested)$^{-1}$. These results demonstrate that larval responses to food levels are not just morphologically plastic, but also physiologically plastic. It is important to further understand these mechanisms of physiological plasticity and their potential link to morphology. Such information will result in a more comprehensive understanding of larval growth and factors that influence recruitment success.

1. Introduction

Planktotrophic marine invertebrate larvae rely on algal food to grow and develop so they can achieve metamorphic competency (Paulay et al., 1985; Strathmann, 1975). Given the potential for food limitation and the overall heterogeneity of the marine environment (Conover, 1968; Huntley and Boyd, 1984; Platt et al., 2003), much research has been directed at understanding how the availability of algal food influences rates of growth and development (e.g., echinoderms (Strathmann et al., 1992); molluscs (Klinzing et al., 2000); crustaceans (Hentschel and Emlet, 2000)). These studies tend to show that high algal abundance leads to faster growth and development and a reduction in planktonic larval duration (PLD), which is advantageous because it minimizes larval exposure to predation or offshore transport away from suitable benthic habitats (Rumrill, 1990).

Studies focused on the planktotrophic larvae of echinoid echinoderms have also revealed a surprising result: echinoid larvae exhibit developmental plasticity, where larval morphology is dependent on algal feeding conditions (e.g., Boidron-Metairon, 1988; Byrne et al., 2008; Hart and Strathmann, 1994; Miner, 2005, 2007; Morgan, 2008; Sewell et al., 2004; Soars et al., 2009). Echinoid larvae exposed to high-food conditions invest fewer resources into ephemeral larval structures (larval arms) and direct more towards post-metamorphic structures such as the rudiment. This results in faster development and a shorter PLD. Larvae in low-food conditions direct more of their limited resources towards increasing the length of the ciliated larval arms to increase algal capture ability (Hart and Strathmann, 1994). This response comes at the expense of slower development of post-larval structures

Abbreviations: DPF, days post-fertilization; PLD, planktonic larval duration; CB, ciliary band; PO, postoral arm; SL, stomach length; AE, assimilation efficiency; GGE, gross growth efficiency; NGE, net growth efficiency; PGE, protein growth efficiency; TH, thyroid hormone

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and a longer PLD. These responses for relatively low- and high-fed larvae are considered adaptive for their respective environmental conditions (Miner, 2011; Strathmann et al., 1992).

In conjunction with research relating food conditions to larval morphology, there are also several studies examining the physiological and biochemical outcomes resulting from different algal feeding conditions. These studies have shown that significant differences in growth performance are linked to changes in protein depositional efficiency (Pace and Manahan, 2007b), lipid composition (Schiopu et al., 2006) and utilization (Adams et al., 2011), as well as energetic partitioning of critical regulatory processes like aerobic energy metabolism (Marsh et al., 1999) and ion regulation (Leong and Manahan, 1997).

Given that echinoid larvae have been such a useful system for understanding developmental plasticity from a morphological perspective, our goal was to further explore the possibility that food availability also exerts plasticity on a physiological level. The occurrence of such physiological plasticity (aka physiological acclimation) has been studied in numerous animals. Temperature-dependent physiological plasticity has been proposed to be a major mechanism by which ectothermic animals survive climate change (Seebacher et al., 2015). Physiological plasticity has also been observed in several contexts including osmotic regulation in fish (Whitehead et al., 2011), social dominance in fish (Maruska and Fernald, 2010), colony structure in coral polyps (Ostrowski et al., 2006), and food and water availability in a mammal (Ostrowski et al., 2006). For the current study, we empirically define physiological plasticity as significant differences in mass-specific rate processes (i.e., mass-specific metabolic rate) and/or energetic efficiencies between low- and high-fed larvae. If physiological plasticity were present during echinoid development, its possible relationship with morphological plasticity could be further explored and considered in the context of the full adaptive value of such plastic responses.

Larvae of the Pacific sand dollar, *Dendraster excentricus*, were fed relatively low and high algal concentrations (1,000 and 10,000 cells mL^{-1}, respectively) and rates of growth (protein and lipid biomass), metabolism (oxygen consumption), and algal ingestion were measured. These values were used to determine mass-specific rates of ingestion and metabolism and for calculation of assimilation efficiency (AE), gross growth efficiency (GGE), net growth efficiency (NGE), and protein growth efficiency (PGE). It was hypothesized that differences in algal abundance would not only cause alterations in important metrics of growth and development, as established by previous studies (e.g., Boidron-Metairon, 1988; Hart and Strathmann, 1994), but also entail significant modifications in physiological state and energetic partitioning related to growth and metabolism. This hypothesis is based on several previous discoveries regarding morphological plasticity in *D. excentricus* larvae: 1) there exists a trade-off between larval arm length and stomach size (Miner, 2005), 2) the biogenic pathways for differential allocation are established prior to the ability to feed (Adams et al., 2011; Miner, 2007), and 3) thyroid signaling can mimic the morphological response to high food conditions (Heyland and Hodin, 2004). That larvae alter allocation to their stomach and arms before feeding is attained suggests important changes are being made in digestive capacity. These changes are likely to have significant impacts on assimilation and growth efficiencies. The involvement of thyroid hormone supports a direct metabolic connection between morphological plasticity and growth energetics (Yehuda-Shnaidman et al., 2014). Previous studies have examined assimilation and growth efficiencies in marine invertebrate larvae (e.g., Almeda et al., 2010; Sprung, 1984; Widdows and Johnson, 1988) as well as empirically determining biochemical energetic expenditures related to growth (Frieder et al., 2016; Pan et al., 2015). However, to the best of our knowledge, none of these approaches have been used to examine developmental plasticity in marine invertebrate larvae.

2. Methods

2.1. Sand dollar collection, spawning, and larval culturing

Adult sand dollars were collected from Los Angeles Harbor in San Pedro, CA (33°42′28.1″ North, 118°16′41.5″ West). Animals were kept in large coolers during transport to the California State University, Long Beach (CSULB) Marine Laboratory. The collected sand dollars were kept in 200 L tanks of flowing seawater at about 16 °C for 24 h or less, before being used for experiments. Coelomic injections of 0.5 M KCl induced gamete release. Sperm were diluted (1:1000) and gently mixed with eggs in sterile-filtered seawater (0.2 μm pore) until achieving a sperm to egg ratio of ~5:1. Fertilization envelopes were counted to confirm successful fertilization (> 90%). For each culture, eggs and sperm from 3 females and 3 males were combined for fertilization, and subsequently separated into different feeding treatments. Due to the need for large numbers of larvae over the length of development studied, larvae were initially cultured at a concentration of 5 individuals mL^{-1}. This concentration decreased during development due to sampling and mortality. While 5 individuals mL^{-1} is a concentration that has been used for many other larval physiology studies (e.g., Pace and Manahan, 2006; Pan et al., 2015; Stumpp et al., 2011; Wheeler et al., 2016), it is higher than that in most studies investigating morphological plasticity (e.g., Boidron-Metairon, 1988; Hart and Strathmann, 1994; Miner, 2007; Sewell et al., 2004). Therefore, for 2 of the 3 cultures used to investigate physiological plasticity, we also measured several aspects of larval morphology to ensure that the occurrence of morphological plasticity was still observed. Seawater in each vessel was completely replaced 3 times per week. During water changes, the entire 20-L of seawater from each vessel were slowly siphoned onto a custom-made Nitex mesh filter (mesh size increased from 50 μm as larvae grew larger). The mesh filter was placed on a 1-L beaker, and excess seawater overflowed from the beaker while the larvae were collected onto the mesh. For a brief period (< 15 min) the larval populations were concentrated and sampled for various measurements, during which time three 100-μL aliquots were taken from each treatment (per day sampled) to estimate the concentration of the entire culture vessel. From the larval concentration, the appropriate volume was calculated to sample the desired number of larvae from the culture. Remaining larvae were then placed into new 20-L vessels (per feeding treatment) of fresh seawater.

An initial, independent experiment was conducted to determine the range of algal concentrations of *Rhodomonas* sp. in which to study physiological parameters during rates of relatively low and high growth in larvae of *D. excentricus*. Fertilized eggs were enumerated and randomly distributed into the following algal feeding treatments: 0; 5,000; 20,000; 40,000; 60,000; and 80,000 algal cells mL^{-1}. Each treatment was held in a 20-L vessel with larvae initially stocked at 5 individuals mL^{-1} in 0.2 μm-filtered seawater. Cultures were held in a temperature-controlled room at 16 °C (± 1 °C) with a 12:12 h light:dark cycle in the CSULB Marine Laboratory. Slowly rotating plexiglass (6–8 rpm) paddles (~18 cm maximum width by 29 cm long) powered by small motors (Buehler Products, Raleigh, NC) kept larvae suspended and algal food homogeneously distributed within cultures. Seawater was replaced three times per week. Algal feeding was initiated at 3 days post-fertilization (DPF) when larvae had developed complete digestive tracts. Larvae were fed the unicellular alga *Rhodomonas* sp. This alga has been used in numerous larval studies and provides a suitable diet for robust growth and development in echinoderm larvae (Schiopu et al., 2006; Strathmann, 1971) and its energetic content has previously been determined (Vedel and Rissgard, 1993). Algae were cultured in Erlenmeyer flasks with sponge stoppers and grown using f/2 media. Algal cultures were always harvested for larval feeding at the end of their logarithmic growth phase. To minimize the potential for microbial growth and other non-specific effects related to algal nutrient media, all media was removed from algae by centrifugation (Beckman Coulter