Measurements on each beaker’s final chl a concentration from larval food limitation experiments conducted on the sand dollar Dendraster excentricus

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Project
» RUI: Effects of large inedible particles on larval feeding, planktonic larval duration, and juvenile quality in marine invertebrates (LIPs on Larval Feeding)

Contributors	Affiliation	Role
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Abstract
Measurements on each beaker’s final chl a concentration from larval food limitation experiments conducted on the sand dollar Dendraster excentricus.

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Coverage

Temporal Extent: 2017-07 - 2019-08

Dataset Description

This dataset contains measurements on each beaker’s final chl a concentration.

Study summary:
In this study of food limitation in the sand dollar *Dendraster excentricus*, we attempted to constrain estimates of the chl a boundary between food-limited and food-unlimited growth by carrying out experiments in nearshore coastal waters of southern California, which frequently have chl a levels >2 ugL-1. We reared larvae in the laboratory in natural seawater collected from the field, as well as in reduced and supplemented food treatments created from this natural seawater. To supplement this investigator-generated variation in food level, we repeated this experiment six times over the course of two years to take advantage of temporal variation in chl a. Within experiments, we assessed whether larvae were food limited by looking both for evidence of food-level induced phenotypic plasticity, and by generating population-level estimates of time to metamorphic competence.

Methods & Sampling

Detailed methods are in a manuscript in review at Marine Ecology Progress Series as of 9 May 2020; additional

details are below. In brief, seawater collected daily was used to prepare three treatments: NS (natural seawater), NS- (NS diluted 1:1 with filtered seawater), and NS+ (NS plus 1000 cells per ml Rhodomonas lens). Treatments were completely changed daily. Initial chl a in each treatment (made in bulk, so only one measure per day) was reported, as was final chl a in each beaker ~24 h after initial. Each beaker's final chl a was measured separately. A, B, C, D refer to the four replicate beakers for each treatment for each day. Beakers were identified by treatment (e.g., NS) and replicate (e.g., A).

RFU was measured with an Aquafluor Handheld Fluorometer (Turner Designs). Means of three measurements of RFU were converted to absolute chl a concentrations using a regression equation generated from calibrations carried out throughout the study.

Detailed Methodology:
Establishing and characterizing treatments
Treatment solutions were prepared using seawater collected each morning from a floating dock near the mouth of Alamitos Bay, in Long Beach, California, USA (33.7458, -118.1151). Seawater was collected from just below the surface in two 9 L carboys. Carboys were transported to the laboratory within the next 20 minutes. On arrival in the laboratory, water temperature (to the nearest 0.1 °C) and salinity (0.1) were measured immediately.

Seawater was held at 16 °C for no more than a few hours until processing. All collected seawater was first filtered through a 35 um mesh into a single 20 L carboy; this removed large inedible particles, as well as potential predators and competitors of D. excentricus larvae. This suspension was mixed, then divided into three 9 L carboys. One of them was designated the natural seawater (NS) treatment. The supplemented treatment (NS+) was created by adding cells of Rhodomonas lens (CCMP739) to another of the carboys to yield a final concentration of 1000 cells per mL (except for the August 2019 experiment, when R. lens was added to yield a final concentration of 5000 cells per mL). The R. lens was cultured in f/2 medium, and before use was pelleted by centrifugation and the medium replaced with seawater filtered through a 0.2 um (pore size) filter (filtered seawater, FSW). The concentration of algal cells in this stock suspension was counted using an Accuri C6 flow cytometer. The reduced food treatment (NS-) was created by diluting the 35-um filtered seawater suspension 1:1 with FSW. After making the treatments, three replicate measurements of chl a fluorescence in each treatment were made with an Aquafluor Handheld Fluorometer (Turner Designs). Absolute chl a concentrations of 1 L samples of treatment solutions were also estimated frequently throughout the study (at least six times for each of the six experiments) using the spectrophotometric method detailed on pp. 193-194 of Strickland & Parsons (1968). An ordinary least squares regression between relative fluorescence units (mean of the three replicates; RFU) and absolute chl a concentration was generated from these data (chl a = 0.232*RFU, R2 = 0.87, n = 40), and later used to convert daily RFU measurements to absolute chl a concentrations.

Obtaining larvae and establishing treatments
Adults of the echinoid Dendraster excentricus were collected from the intertidal and shallow subtidal zones near San Pedro, California (33.7078, -118.2766) and maintained in recirculating seawater tanks at 16 °C in the laboratory for up to one month before use. We obtained gametes from adults by injecting ~1 mL of 0.53M KCl into the perivisceral coelom. Eggs were rinsed once with FSW, resuspended in ~50 ml of FSW, then fertilized using diluted sperm. Fertilized eggs were then transferred to 1 L of FSW, and held unstirred in an environmental chamber at 16 °C. By 24 h post-fertilization (1 dpf), blastulae had hatched and congregated at the water surface. Swimming blastulae were then decanted into a clean beaker. We estimated the concentration of blastulae in this stock suspension in five 500 ul subsamples, and used the mean of these estimates to calculate the volume of stock suspension needed to deliver 250 larvae to each replicate beaker (0.25 larvae per mL). We added this volume to each of the 12 experimental 1 L beakers (three treatments, each replicated four times), each filled with the appropriate treatment solution (in the July 2017 experiment, there were only three replicates per treatment). Beakers were then placed on a paddle stirrer in an environmental chamber maintained at 16 °C.

Beginning in the April 2018 experiment, we used the methods above to add an estimated 250 larvae to each of two or three additional 1 L beakers of FSW to which 10,000 cells per mL of Rhodomonas lens had been added. These "count control" beakers were maintained on a paddle stirrer at 16 °C for 3-4 days with no water changes, during which time larvae grew substantially and became much easier to count. The larvae in each of these beakers were then concentrated onto a 65 um mesh, resuspended in ~50 mL FSW, killed with formalin, then counted in a Bogarov tray. Our expectation was that most mortality in our treatment beakers was associated with the stress of water changes; in the absence of water changes, as in the count control beakers, we expected very little mortality. This method allowed us to characterize how closely we approached our target initial concentrations of larvae in each experiment, and to generate more accurate estimates of mortality in each treatment beaker.

Daily maintenance
Treatments were established at 1 dpf (~24 h after fertilization). Because at this stage of development larvae were small and fed at low rates, we did not change water and reestablish treatment conditions until 3 dpf, but after 3 dpf we carried out water changes daily until termination of the experiment. On each of these days, we first estimated the amount of chl a remaining in each beaker using the fluorometer. We then did a complete water change in each beaker by "forward filtration". Larvae in each beaker were concentrated on to a 120 um mesh and their original beaker cleaned using hot freshwater. Larvae were then rinsed back into their original beaker using the appropriate treatment suspension and the beaker was replaced on the paddle stirrer at 16 °C until the next day's water change. Because treatments were based on natural seawater passed through a 35 um mesh, but daily water changes involved discarding all beaker contents that passed through a much larger mesh (120 um), any competitors (primarily rotifers) that were seeded into beakers on a given day were completely removed the next day, minimizing their effects on larvae.

The only exceptions to this pattern of daily maintenance came in the February 2019 experiment, during which rain events lowered the salinity of raw seawater to 25 at 9 dpf, and 28 at 13 dpf. On both of these days, we did not carry out water changes to avoid exposing larvae to low salinity water. Instead, on those two days we simply supplemented the contents of all treatment beakers with sufficient stock suspension of R. lens to provide an additional 1000 cells per mL. This concentration of R. lens was chosen with the intent of bringing initial chl a concentrations on those two days back within the range of initial chl a concentrations in the NS treatment in prior days of that experiment. At 10 and 14 dpf, salinity had returned to normal or nearly so (33 and 30, respectively), and we resumed normal water changes.

Larval form
At 7 dpf, during normal maintenance, we removed ~8-10 larvae from each experimental beaker and placed them in a drop of seawater on a glass slide. Larvae were briefly relaxed in a 1:1 solution of seawater and 7.5% $MgCl_2$, then killed by addition of dilute formalin. An eyelash probe was used to orient each larva ventral side up, and a coverslip supported by Plasticene modelling material was placed over them. Larvae were observed using an Olympus BX-51 compound microscope. The first five correctly oriented larvae encountered on the slide were imaged using a QIClick monochrome camera. A stage micrometer was also imaged. We later opened these images in ImageJ 2.0.0 to estimate the length of the right postoral arm (from the ventral transverse rod to its tip) and midline body length (Fig. S1). Only the right postoral arm was measured to avoid between-arm variation due to directional asymmetry.

Time to metamorphic competence
Beginning at 10 dpf, during daily water changes we removed ~10-15 larvae from each experimental beaker and placed them in 2 mL of FSW in a well of one of two six-well tissue culture plates. After all experimental beakers had been processed, we added 2 mL of a solution of FSW with 80 mM excess KCl to each well, yielding a final concentration of 40 mM excess KCl. The plates were incubated at 16°C for 1 hour, and then individuals in each well were scored for metamorphosis. Individuals were scored as metamorphosed when the tissue on each larval arm had pulled back from the tips of the arms and coalesced in a ball at the posterior end of the larva. Larvae that had not metamorphosed had not changed in form at all. The number of metamorphs and unmetamorphosed larvae were recorded. This process was repeated daily for each of the experimental beakers. When the samples of larvae from all beakers in a given treatment reached ≥50% metamorphosis for two days in a row, the remaining larvae in each of the beakers of that treatment were concentrated on to a mesh, rinsed into a 50 mL centrifuge tube, preserved with dilute formalin, and later counted in a Bogorov tray.

Larval mortality
For four of the six experiments we had independent estimates of the initial number of larvae in each beaker. We had also recorded the number of larvae removed from each beaker for imaging of larval form and estimating time to metamorphic competence, and the number of larvae remaining in each beaker when the treatment was terminated. These values allowed us to estimate instantaneous mortality rates in each beaker. Mortality rates were estimated by solving for m in the equation $N_t = N_0 e^{-mt}$, where N_t is the total number of larvae accounted for in a beaker, N_0 is the initial number of larvae in a beaker, m is instantaneous mortality (d^{-1}), and t is the length of the experiment (d).

Analysis
Morphological responses to food concentration in echinoid larvae are often estimated by comparison of the ratio of postoral arm length to body length. We compared the average within-beaker ratio of right postoral arm length to body length among treatments for each experiment using one-way ANOVA in Prism 8. All larval form data met assumptions of normality and homoscedasticity, as assessed with Shapiro-Wilk and Bartlett's tests, respectively. Where ANOVA indicated significant differences among treatments, we used post hoc Dunnett's tests to make the two comparisons of interest, between the NS+ and NS treatments, and the NS- and NS treatments. Time to 50% metamorphic competence (TC50) for each beaker in each experiment was estimated

by logistic regression using R 3.6.2 (R Core Team 2019). TC50 was compared among treatments for each experiment using one-way ANOVA, with post hoc tests as above. Assumptions of normality and homoscedasticity were met for all TC50 data except for February 2019, where a Shapiro-Wilk test indicated that data were not normally distributed. We did not transform data for that month, however, since visual examination of the quantile-quantile plot suggested that deviations from normality were minor, and because one-way ANOVA is typically robust to even considerable deviations from normality.

Data Processing Description

BCO-DMO Processing:
- concatenated data from separate files;
- renamed fields.

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Data Files

File
chl_final.csv (Comma Separated Values (.csv), 63.24 KB) MD5:d89dfc8c592f908fb53e6bac2770fce2 Primary data file for dataset ID 819293

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Parameters

Parameter	Description	Units
month_year	Month and year	unitless
Day	Collection day (sequential, e.g. "7" is the 7th day of that experiment); Day 0 for each experiment was spawning and fertilization of sand dollars, into filtered seawater; Day 1 was the first day of collection of natural seawater.	unitless
treatment	Treatment and beaker. Treatments: NS = natural seawate), NS- = NS diluted 1:1 with filtered seawater, and NS+ = NS plus 1000 cells per ml Rhodomonas lens. A, B, C, D refer to the four replicate beakers for each treatment.	unitless
sampling_time	Time of sampling	unitless
final_RFU_1	Measurement of relative fluorescent units	arbitrary
final_RFU_2	Measurement of relative fluorescent units	arbitrary
final_RFU_3	Measurement of relative fluorescent units	arbitrary
mean_final_RFU	Mean of the three RFU measurements	arbitrary
final_chl	Final chl calculated using a regression equation generated from calibrations carried out throughout the study.	micrograms per liter (ug/L)
Treatment_mean	Treatment mean chl	micrograms per liter (ug/L)
pcnt_removed	Percent chl removed from initial conditions	unitless (percent)
min_chl_a	minimum chl a	micrograms per liter (ug/L)
max_pcnt_chl_a_removed	maximum percent chl a removed from initial conditions	unitless (percent)
max_chl_a	maximum chl a	micrograms per liter (ug/L)
min_pcnt_chl_a_removed	minimum percent chl a removed from initial conditions	unitless (percent)

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Instruments

Dataset-specific Instrument Name	Aquafluor Handheld Fluorometer
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

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Project Information

RUI: Effects of large inedible particles on larval feeding, planktonic larval duration, and juvenile quality in marine invertebrates (LIPs on Larval Feeding)

Coverage: Southern California Bight

NSF award abstract:
Many ecologically and economically important marine invertebrates (e.g., oysters, crabs, and sea urchins) have life cycles that include feeding larval stages that live drifting in the water as part of the plankton. These larvae spend days or weeks feeding on tiny algal particles to fuel their development until they can metamorphose into juveniles. In nature, however, the plankton includes not only edible particles, but also many particles that are too large to be eaten but which may interfere with feeding on edible particles. These include, for example, large algal particles, eggs and embryos of other invertebrates, re-suspended sediment, and anthropogenic nano- and micro-plastics. When larvae encounter large inedible particles, they may respond by altering their swimming behavior to avoid them, or by capturing and then rejecting them. Such interactions reduce the rate at which larvae can capture edible particles, which forces them to either spend more time feeding before metamorphosis (increasing their overall risk of dying due to planktonic predators), or to metamorphose with less energy, producing juveniles in relatively poor condition. This project examines how large inedible particles affect feeding, time to metamorphosis, and juvenile condition in the larvae of diverse marine invertebrates. The project has the potential to dramatically change our understanding of how larvae feed and survive in natural communities, and thus our understanding of the population dynamics of these important organisms. The project will support research training opportunities for undergraduate and graduate students at California State University Long Beach, a primarily undergraduate institution, as well as summer research internships for students at two local community colleges. Project data will be integrated into laboratory modules in undergraduate courses. Finally, data on the reproductive biology of diverse California marine invertebrates will be added to a public website that is widely used by members of the public, students, and biologists interested in the development, life histories, ecology, and evolution of these common animals.

The factors that control planktonic duration and juvenile condition in marine invertebrates with feeding larvae have long been recognized as critical to understanding their ecology and evolution. Larval feeding environment is clearly one of those factors, but previous work has focused almost exclusively on one feature of that environment, the abundance of food. This project will evaluate the importance of another potentially critical dimension of the larval feeding environment: the presence of large inedible particles, which are frequently abundant in natural plankonic communities. It takes a comparative approach to address two key questions about the effects of large inedible particles on larvae (including those of echinoderms, annelids, and molluscs) that feed using several different particle capture mechanisms. First, do large inedible particles present in natural plankton reduce larval feeding rates? And second, does the presence of large inedible particles extend larval planktonic duration or result in the production of lower quality juveniles? Feeding rates of larvae will be measured in short-term experiments in which larvae are exposed to both food and to natural or artificial large inedible particles over a range of concentrations. Effects of large inedible particles on planktonic duration and

juvenile quality will be measured by culturing larvae through their entire life cycles in the presence of large inedible particles at various concentrations. Because feeding performance is an important determinant of planktonic duration, larval survival, and juvenile condition, the project will add greatly to our understanding of how conditions in the plankton affect the population dynamics of the many marine invertebrates with feeding larvae.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756531

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