Phenotypic plasticity of the ciliated band of seven species of echinoderm larvae, collected between 2020 and 2022 in the laboratory at California State University, Long Beach.

Website: https://www.bco-dmo.org/dataset/914002
Data Type: experimental
Version: 1
Version Date: 2024-04-10

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
This experiment assesses phenotypic plasticity of the ciliated band. This dataset contains estimates of the ciliated band generated by both direct measurement and by tracing of the band for non-pluteus larvae, used in evaluation of tracing as a proxy for direct measurement. The data was collected between 2020 and 2022 in the laboratory at California State University, Long Beach.

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Coverage

Location: Southern California Bight
Spatial Extent: **N**:33.7666 **E**:-118.091 **S**:33.6808 **W**:-118.427
Temporal Extent: 2020-01 - 2022-06

Dataset Description

Study Summary: The feeding larvae of echinoderms take two distinct forms: plutei (echinoids, ophiuroids), which have calcified skeletal rods supporting long, slender arms bearing the ciliated band, and non-plutei (asteroids, holothuroids), where the ciliated band is borne on rounded lobes of tissue that do not contain skeletal rods. Feeding larvae of all four classes of echinoderms are known to alter the length of their ciliated bands in response to food ration, with larvae fed low rations producing longer ciliated bands relative to body size than larvae fed high rations. However, phenotypic plasticity of the ciliated band has been studied much less in non-plueti and comparisons among classes are difficult since prior studies vary in methods. We sought to determine how the plutei and non-plutei compared in their expression of plasticity in the ciliated band using standardized conditions for seven species (four plutei, three non-plutei). We measured the length of the ciliated band and body length at three developmental timepoints, comparing larvae provided high (6000 cells

ml^{-1} *Rhodomonas lens*) and low (1000 cells ml^{-1}) food rations.

Methods & Sampling

Collection of adults and spawning: Adults of each species were collected from intertidal or shallow subtidal zones from various sites in Los Angeles County and transported to California State University Long Beach, where they were maintained in recirculating seawater tanks at 16 °C until their use in experiments. Experiments were carried out on one species at a time, depending on reproductive seasonality for that species.

Spawning was induced using standard methods (e.g., M. Strathmann, 1987). The echinoids *Dendraster excentricus*, *Lytechnius pictus*, and *Strongylocentrotus purpuratus* were induced to spawn via injection of 0.2-1.0 mL (depending on adult size) 0.53 M KCL into the perivisceral coelom. The asteroids *Patiria miniata* and *Astropecten armatus* were induced to spawn by injection of 1-3 mL 100 µM 1-methyladenine. The holothuroid *Apostichopus parvimensis* was injected with 3 mL of 200 µM NGLWY-amide (Kato et al., 2009). The ophiuroid *Ophiothrix spiculata* was exposed to 4 °C water in the dark for 15 minutes, then to room temperature water and sunlight for 15 minutes; this treatment was repeated for up to two hours (Selvakumaraswamy & Byrne, 2000). For all species, adults were each induced to spawn in their own isolated containers, allowing us to control subsequent fertilizations.

For each species, we prepared four full-sibling families, each with a unique pair of parents. Gametes were collected from four parents of each sex. Eggs of each female were rinsed and resuspended in FSW. Two to four concentrated drops of sperm from each male were diluted in 50 mL FSW. The diluted sperm of a single male were then added to the rinsed eggs of a single female a few drops at a time until fertilization approximated 90%. Fertilized eggs were then diluted with FSW and allowed to develop undisturbed for 24-30 h at 16 °C. By that time embryos had reached the swimming blastula or gastrula stages (depending on species) and were concentrated at the water's surface. We decanted the embryos of each unique family into a clean beaker, stirred them well, then estimated the concentration of embryos in each of these stock suspensions (each representing a unique family) by averaging the number of larvae in five 0.5 mL subsamples. For each species, we sought to expose larvae of four unique families to low and high food treatments in a paired (by family) design. Thus, for each of the four unique families, we prepared two beakers, each containing 1 L of FSW. To each of these beakers we added the appropriate volume of stock suspension to deliver an estimated 250 embryos from that family.

Culturing: One beaker per family was fed the low food ration (1000 cells mL−1 *Rhodomonas lens*) and the other was fed the high food ration (6000 cells mL−1 *R. lens*). This resulted in eight beakers per species, with each low-fed beaker paired with a high-fed beaker of the same family. *Rhodomonas lens* were isolated from their growth medium via centrifugation, resuspended in FSW, and counted using a BD Accuri C6 flow cytometer. Cultures were maintained in the 16 °C environmental chamber and continuously stirred by a paddle system (Strathmann, 1987). Daily water changes began on the third day post fertilization (dpf), allowing larvae to develop without disturbance for a day while still in or just completing the pre-feeding period. To change the water, cultures were filtered through a 60 µm sieve to capture larvae. The sieve was submerged in shallow water while filtering so larvae were not exposed to air. Larvae were then gently rinsed from the filter with fresh FSW back into their cleaned beakers and fed.

Sampling: We imaged larvae at three developmental timepoints: just prior to the onset of their ability to feed ("pre-feeding"), and at two timepoints after the onset of feeding ("early" and "mid"). The timing of pre-feeding, early, and mid-development measurements varied among each of the species depending on their rate of development. For pre-feeding measurements, we aimed to sample larvae just before the onset of feeding in the culture. These timing of the onset of feeding was determined from prior observations (e.g. Pernet et al., 2017), or, in cases where prior observations of development at similar temperatures were not available, by rearing a preliminary culture in the same conditions as the main experiment, checking larvae in that culture for evidence of feeding (algal cells in the stomach) at least three times daily.For the later timepoints, we aimed to measure larvae before there was visible evidence of a rudiment in echinoids and asteroids. For most species, 7 dpf was the default day for early-development images, and 14 dpf was the default day for mid-development measurements. These measurements were taken earlier in echinoids due to their rapid development, the most extreme being 3 dpf and 7 dpf for *D. excentricus*, followed by 5 dpf and 10 dpf for *L. pictus*, and 6 dpf and 12 dpf for *S. purpuratus*. Despite these precautions we observed that many *D. excentricus* had begun rudiment development by 7 dpf, and some *S. purpuratus* were at the very earliest stages of rudiment invagination (as described by Heyland & Hodin, 2014) at 12 dpf.

At these timepoints, we measured ciliated band length as described above. Body length was also estimated from two landmarks. Three additional parameters – mouth width, stomach length, and stomach width – were measured in two dimensions using a single image from the stack in which the structure of interest was best in focus. The geometric mean of stomach length and width was used to represent stomach size (Strathmann, 2022). Additionally, in bipinnaria and auricularia larvae, the ciliated band was traced from a single image as depicted in Caballes et al. (2017) to evaluate it as a proxy for the direct measurement of the ciliated band length.

Data Processing Description

ImageJ 1.53a used to determine x, y, and z coordinates of biometric landmarks of larvae from the image stacks. Excel was used to calculate total distances of biometric measurements from x, y, z coordinates determined in ImageJ.

BCO-DMO Curation Notes

* Changed species names to full species names: Astropecten armatus, Apostichopus parvimensis, Dendraster excentricus, Lytechinus pictus, Ophiothrix spiculata, Patiria miniata, Strongylocentrotus purpuratus
* Adjusted parameter names to comply with database requirements

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Data Files

File
914002_v1_plasticity.csv (Comma Separated Values (.csv), 185.34 KB) MD5:20306372338213fee5a45d5003cbe074
Primary data file for dataset ID 914002, version 1

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Related Publications

Caballes, C., Pratchett, M., & Buck, A. (2017). Interactive Effects of Endogenous and Exogenous Nutrition on Larval Development for Crown-Of-Thorns Starfish. Diversity, 9(1), 15. https://doi.org/10.3390/d9010015
Methods

Heyland, A., & Hodin, J. (2014). A detailed staging scheme for late larval development in Strongylocentrotus purpuratus focused on readily-visible juvenile structures within the rudiment. BMC Developmental Biology, 14(1). https://doi.org/10.1186/1471-213x-14-22 https://doi.org/10.1186/1471-213X-14-22
Methods

Kato, S., Tsurumaru, S., Taga, M., Yamane, T., Shibata, Y., Ohno, K., Fujiwara, A., Yamano, K., & Yoshikuni, M. (2009). Neuronal peptides induce oocyte maturation and gamete spawning of sea cucumber, Apostichopus japonicus. Developmental Biology, 326(1), 169–176. https://doi.org/10.1016/j.ydbio.2008.11.003
Methods

Selvakumaraswamy, P., & Byrne, M. (2000). Vestigial ophiopluteal structures in the lecithotrophic larvae of Ophionereis schayeri (Ophiuroidea). The Biological Bulletin, 198(3), 379–386. https://doi.org/10.2307/1542693
Methods

Steiner, B., Syveurud, A., Pernet, B. (2024). Scope for phenotypic plasticity in ciliated band length in echinoderm larvae. Manuscript in review.
Results

Strathmann, M. F. (1987). Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast: Data and Methods for the Study of Eggs, Embryos, and Larvae. University of Washington Press.

Strathmann, R. R. (2022). Scope for Developmental Plasticity of Feeding Larvae of a Holothuroid, Contrasted with Other Echinoderm Larvae. The Biological Bulletin, 242(1), 1–15. https://doi.org/10.1086/717157
Methods

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Parameters

Parameter	Description	Units
Form	Larval form as either pluteus or non-pluteus	unitless
Species	Echinoderm species	unitless
Age	Age of larva in days post fertilization	days
Dev	Relative developmental stage of larva	unitless
Treatment	Feeding treatment; with High indicating 6k algal cells/mL and Low indicating 1k algal cells/mL	unitless
Family	Identifies the family the larva belongs to. Each family consists of full-siblings from a unique mother and father cross	unitless
Larva	Identifying number for the larva. Five larvae were measured per timepoint. In rare instances the identifying value is 6 if one of the first 5 larvae encountered had an unsatisfactory image.	unitless
cbl	Total length of the ciliated band measured in three dimensions from image stack	mm
bl	Body length measured in three dimensions from image stack	mm
sl	Stomach length measured from single image	mm
sw	Stomach width measured from single image	mm
sgmean	Geometric mean of the stomach length and width	mm
mpo	Mean postoral arm length measured from transverse rod. Only applicable to plutei	mm

po1	Length of left postoral arm. Only applicable to plutei	mm
po2	Length of right postoral arm. Only applicable to plutei	mm
mouth	Mouth width	mm
trace1	Length of the pre-oral lobe measured by tracing. Only applicable to non-plueti	mm
trace2	Length of the ciliated band (excluding the pre-oral lobe) measured by tracing. Only applicable to non-plueti	mm
trace_full	Length of the full ciliated band measured by tracing. Calculated by addition of trace1 and trace2. Only applicable to non-plueti	mm
geospatial_bound_N	The northern latitude of the bounding box that includes the collection site of all adult specimens	decimal degrees
geospatial_bound_S	The southern latitude of the bounding box that includes the collection site of all adult specimens	decimal degrees
geospatial_bound_E	The eastern longitude of the bounding box that includes the collection site of all adult specimens	decimal degrees
geospatial_bound_W	The western longitude of the bounding box that includes the collection site of all adult specimens	decimal degrees
experiment_location_lat	The latitude of the laboratory location where experiments took place	decimal degrees
experiment_location_long	The longitude of the laboratory location where experiments took place	decimal degrees
experiment_start	The year and month (ISO format, yyyy-mm) when the first larval experiment began	unitless
experiment_end	The year and month (ISO format, yyyy-mm) when the final larval experiment concluded	unitless

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Instruments

Dataset-specific Instrument Name	BD Accuri C6 Flow Cytometer (BD Biosciences)
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	BD Accuri C6 Flow Cytometer (BD Biosciences) was used to determine concentration of Rhodomonas lens for larval feeding.
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. Description from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm

Dataset-specific Instrument Name	
Generic Instrument Name	Fluorescence Microscope Image Analysis System
Dataset-specific Description	Olympus BX-51 compound microscope outfitted with a QIClick camera (Teledyne Photometrics) and a motorized z-axis drive, both controlled via MicroManager software to create image stacks of fixed larvae for biometric measurements.
Generic Instrument Description	A Fluorescence (or Epifluorescence) Microscope Image Analysis System uses semi-automated color image analysis to determine cell abundance, dimensions and biovolumes from an Epifluorescence Microscope. An Epifluorescence Microscope (conventional and inverted) includes a camera system that generates enlarged images of prepared samples. The microscope uses excitation ultraviolet light and the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light.

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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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