Quantification of ciliated band length per unit protein in early echinoderm larvae (protein data), collected between 2020 and 2022 in the laboratory at California State University, Long Beach.

Website: https://www.bco-dmo.org/dataset/914146
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 compares the ciliated band length to protein content of eight species and the protein content of the larvae. The data was collected between 2020 and 2022 in the laboratory at California State University, Long Beach.

Table of Contents

Coverage

Location: Southern California Bight
Spatial Extent: **N**:33.766 **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. Prior work suggests that the structural cost of adding a given length of ciliated band might be lower for plutei than for non-plutei, which might affect the scope for phenotypic plasticity in ciliated band length in the two types of larvae.

In this study we test the hypothesis that plutei and support a greater length of ciliated band per unit biomass than non-plutei by comparing ciliated band length and protein content of larvae of eight species (with at least one species from each echinoderm class that includes feeding larvae) at two timepoints in early development.

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*, *Strongylocentrotus purpuratus* and *S. fragilis* 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. Spawning continued until a minimum of three parents of each sex were obtained. Sperm from each spawning male was combined with eggs from each spawning female. Once the larvae reached the swimming stage (~24 hours), the offspring of all parents were combined to produce a genetically diverse population.

Culturing: Larvae were distributed into seven replicate 2 l beakers, with a total of 500 larvae per beaker (for *S. purpuratus*, 14 beakers of 500 larvae each were produced since greater numbers were needed for their protein analysis due to their small size). Larvae were fed 6000 cells ml−1 *Rhodomonas lens* which 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: For all species except *D. excentricus*, sampling for images and protein analysis occurred at 5 (“early-development”) and 10 dpf (“mid-development”). Sampling for *D. excentricus* occurred at 3 and 5 dpf due to their more rapid development and early formation of the rudiment. While larvae were concentrated during the water change, we collected larvae using a pulled pipette. Depending on larval size and age, three samples of 30-80 larvae each were collected from each beaker and placed in 1.5 ml microcentrifuge tubes on ice. The remaining larvae after the early-development sampling were returned to their beaker and FSW was added to approximate a larval concentration of 0.25 larvae ml−1 based on an estimation of remaining larvae. Both *Strongylocentrotus* species introduced small variations to these methods, since their small size necessitated experiments with greater numbers. Thus, for *S. purpuratus*, 14 replicate beakers were made on 1 dpf so three samples of 150 larvae each could be taken on 5 dpf from seven of the beakers (and the remaining larvae in those beakers discarded), and the other seven beakers were used for the mid-development samples of 80 larvae each. *S. fragilis* was included in this study opportunistically, so we were not able to repeat the experiment with greater numbers and instead had fewer replicates for *S. fragilis* than for the other species. *S. fragilis* had a total of 6 replicate cultures instead of 7, and the 10 dpf timepoint consisted of only two protein samples rather than three due to a shortage of larvae.

The tubes containing larvae sampled for protein analysis were centrifuged to pellet the larvae. Water was aspirated gently using a pipette pulled to a tip size of 0.2 mm. Once aspiration was complete, the samples were stored at -80 °C for up to three months.

Protein Quantification: To process the protein samples, we added 100 µl of 4 °C Nanopure water to each sample, and placed them on ice. The larval cells were then lysed using Fisherbrand polypropylene pestles designed to fit 1.5 ml microcentrifuge tubes. A Thermo Scientific Micro BCA Protein Assay Kit was used to determine the protein content of samples against a BSA (Bovine Serum Albumin) standard curve. 376 µl of 4 °C Nanopure water to each to provide adequate volume for the assay. The samples were vortexed for 10-15

seconds. 150 µl from the homogenized sample was then placed into three experimental wells of a 96-well plate to produce three technical replicates per sample. After addition of reagents and incubation according to the manufacturer's instructions, the plates were read using a Biotek Synergy H1 Hybrid Microplate Reader. To calculate protein content per individual, the number of larvae per technical replicate was calculated as: larvae in technical replicate = larvae in original sample * (150 µl technical replicate volume)/(476 µl sample volume).

Data Processing Description

Protein: Excel was used to generate the BSA standard curve for conversion of spectrophotometric absorption values to protein content. Two BSA standard ladders were produced per plate. The R2 value of the standard curve exceeded 0.98 each time the assay was performed. The sample readings were then calculated as ng protein per individual for each technical replicate, which were then averaged to yield a single value per sample. This resulted in three larval protein content estimates for each beaker at each developmental timepoint.

BCO-DMO Processing Description

* 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

[table of contents | back to top]

Data Files

File
914146_v1_protein.csv (Comma Separated Values (.csv), 43.73 KB) MD5:e1d4a205e6c0ffd12b14da950f56ea56 Primary data file for dataset ID 914146, version 1

[table of contents | back to top]

Related Publications

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. http://www.jstor.org/stable/j.ctvcwnh8b
Methods

[table of contents | back to top]

Related Datasets

IsRelatedTo

Steiner, B., Pernet, B. (2024) **Quantification of ciliated band length per unit protein in early echinoderm larvae (biometirc data), collected between 2020 and 2022 in the laboratory at California State University, Long Beach.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-04-10 doi:10.26008/1912/bco-dmo.914147.1 [view at BCO-DMO]
Relationship Description: Part of the same experiment comparing ciliated band length to protein content of echinoderm species. This files contains the biometric data.

[table of contents | back to top]

Parameters

Parameter	Description	Units
Species	Echinoderm species	unitless
Age	Age of larva in days post fertilization	days
Dev	Relative developmental stage of larva	unitless
Beaker	Beaker that larva was reared in unique for each species	unitless
Sample	Identifying number for protein sample. Unique for each Species age and beaker combination	unitless
Average	Average protein content per larva in that sample	nanograms (ng)
sd	Standard deviation for the protein content estimate for each sample based on three technical replicates	unitless
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

[table of contents | back to top]

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. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	Biotek Synergy H1 Hybrid Microplate Reader
Generic Instrument Name	plate reader
Dataset-specific Description	Biotek Synergy H1 Hybrid Microplate Reader, to measure spectrophotometric absorption values of protein samples and BSA standards.
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

Dataset-specific Instrument Name	Thermo Scientific Micro BCA Protein Assay Kit
Generic Instrument Name	Protein Assay Kit
Dataset-specific Description	Thermo Scientific Micro BCA Protein Assay Kit used to determine protein content of samples against a bovine serum albumin (BSA) standard curve.
Generic Instrument Description	The Pierce Micro BCA Protein Assay Kit is a three-component version of our BCA reagents, optimized to measure total protein concentration of dilute protein solutions (0.5 to 20 µg/mL).

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

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

[table of contents | back to top]