Body length, right postoral rod length, and stomach length of Dendraster excentricus and Lytechinus pictus larvae raised at three culture densities on two food rations from 2021-2022 (LIPs on Larval Feeding project)

Website: https://www.bco-dmo.org/dataset/879120
Data Type: experimental
Version: 1
Version Date: 2022-08-24

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
The feeding larvae of many echinoids develop long postoral arms relative to body length when food is sparse but relatively short postoral arms when food is abundant, a response thought to adaptively adjust feeding capability. However, in an important recent study, larvae of Dendraster excentricus exhibited this food-conditioned plasticity only when reared at a high density typical of laboratory cultures; when reared at a lower density more representative of larval densities in nature they did not exhibit this plastic response. This suggests that laboratory results cannot be easily extended to make inferences about phenotypic plasticity in nature. We replicated this study and extended it to an even lower larval culture density and to a second species, Lytechinus pictus. Larvae of D. excentricus developed longer arms adjusted for body length when fed the lower of two food rations at all culture densities, though differences were only marginally significant at the lower culture density in one experiment. Larvae of L. pictus tended to develop longer arms adjusted for body length at lower food rations, though differences only approached statistical significance at the highest culture density in one experiment. For both species, contrasts between food rations almost always showed an inverse relationship between postoral arm length and stomach length, consistent with prior work demonstrating trade-offs in investment in these two features characteristic of phenotypic plasticity. The data submitted here were collected in 2021 and 2022 in the laboratory at California State University, Long Beach. Two datafiles are provided: one containing data on actual densities in culture vessels (to check on treatment effectiveness: density_counts.csv), and one containing all the larval morphology data (density_morphometrics.csv). In addition, we provide R code used to produce the analyses in a paper describing this work (Nilsson P, Pernet B. (In press, 2022) Echinoid larvae can express food-conditioned morphological plasticity at ecologically relevant culture densities. Mar Ecol Prog Ser).

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Coverage

Temporal Extent: 2021-01 - 2022-04

Methods & Sampling

Methodology:

Detailed methods are presented in a manuscript in press at Marine Ecology Progress Series as of 27 June 2022 (Nilsson & Pernet (2022). A brief summary of the larval culture and counting methods are presented below.

Adult echinoids (*Dendraster excentricus* and *Lytechinus pictus*) were collected from two sites near San Pedro, California and spawned in lab to yield three full-sibling families per experiment. In each experiment, blastulae were allowed to develop for one day, then distributed to beakers. Ten beakers were prepared for each of three culture density treatments (0.015, 0.05, and 0.25 larvae ml-1). Half (five) of these beakers were allocated a daily ration of *Rhodomonas lens* (CCMP739) at concentrations of 250 cells ml-1 and the other half (five) allocated 5,000 cells ml-1 to establish two food ration treatments; all received daily water changes.

In each experiment, we assessed the accuracy of larval aliquoting by preparing three additional count control beakers for the 0.05 and 0.25 larvae ml-1 treatments as described above. Larvae in these beakers were fed the higher (5,000 cells ml-1) food ration and cultured at room temperature to accelerate their development, increasing their size and opacity and making them easier to count accurately. At 3 days post fertilization (dpf), the larvae in each count control beaker were concentrated into a small (5 – 20 ml) volume and killed with ethanol. They were then counted to estimate the actual number of blastulae that had been delivered to the experimental beakers at each density. No count control beakers were prepared for the 0.015 larvae ml-1 treatment because those beakers were populated by counting out and pipetting the exact number of desired larvae (15) to each beaker.

Sampling:

The mean larval count for each treatment was compared to the expected number of larvae; as all beakers contained 1000 ml, the expected numbers were 50 and 250 for the 0.05 and 0.25 larvae ml-1 treatments respectively.

These larval counts can be found attached to this metadata record below Data Files within the file, larvae_density_counts.csv.

Analysis:

This analysis was reproduced from Nilsson & Pernet (2022). Studies on feeding structure plasticity in echinoids use diverse statistical approaches in their analyses (McAlister & Miner 2018), a fact that exacerbates the difficulty of comparing results among studies which are already diverse in experimental technique. To enable straightforward comparison with the results of Kacenas & Podolsky (2018), we used the same statistical approach that they did, creating linear mixed-effects models for both dependent variables (PORL and SL) for each experiment. Kacenas & Podolsky (2018) did this using IBM SPSS 24 (IBM Corp. 2016), but we did our analyses in R 4.1.0 (R Core Team 2021) using the lmer function provided by the lme4 1.1-27 package (Bates et al. 2015) and extended by the lmerTest 3.1-3 package (Kuznetsova et al. 2017). Food ration and larval density were treated as fixed effects, beaker as a random effect, body length as a covariate, and either postoral rod length or stomach length as response variables. Estimated marginal means were calculated in R using the emmeans package (Lenth 2021) and compared using the glht function of the multcomp package (Hothorn et al. 2008). To verify that any differences in results were not due to differences between software packages in the implementation of statistical routines, we also analyzed the data from *D. excentricus* Expt 1 using IBM SPSS 24 (IBM Corp. 2016). Similar values were produced by both packages.

To ensure our statistical conclusions were not particular to the approach that both we and Kacenas & Podolsky (2018) used, we conducted several additional analyses, including similar linear mixed-effects models that included covariate interaction terms as well as a simpler approach using ANOVA to compare PORL and SL adjusted for larval size by dividing each by BL. These analyses, which are detailed in the Supplementary Material, all produced results similar to those of our primary analysis (Tables S1-S5, Fig. S1).

Data Processing Description

Processing notes from Researcher:

The morphometric data (Midline_body_length, Right_PO_arm_length and Stomach_length) were measured with the Fiji distribution (2.3.0/1.53f51) of ImageJ (Schindelin et al. 2012). The exact landmarks used to define these measurements are presented in Figure 1 of Nilsson & Pernet (2022). These data were subsequently analyzed as described in Nilsson & Pernet (2022).

The code required to reproduce these analyses is available at https://doi.org/10.5281/zenodo.6824313.

Problems/Issues Notes from Researcher

For the second experiment on Dendraster excentricus (“D. excentricus Expt 1”), the count control beakers for both the 0.05 and 0.25 ml-1 larval densities yielded approximately half the expected number of larvae; we view this as a conservative error, providing a stronger test of the ability of larvae to exhibit plasticity at low culture densities than the intended concentrations. As we hand-counted into the 0.015 larvae ml-1 treatments, those treatments could not have been affected by inaccuracies in aliquoting. See the associated density_counts dataset for details.

For the 0.015 larvae ml-1 density treatment in both Lytechinus pictus experiments (“L. pictus Expt 1” and “L. pictus Expt 2”), there were often fewer than five larvae that could be measured, due to a combination of low survival and high incidence of larvae with missing or obviously broken right postoral rods. Low survival and abnormal arm morphology past 5 dpf was also noted in a study on culture methods for larvae of the congener Lytechinus variegatus (Buitrago et al. 2005), which are known to be particularly fragile and thus likely to be damaged during water changes (Lowe & Wray 2000).

Note that the lowest culture density treatment (0.015 larvae ml-1) was only introduced beginning in the second experiment, and thus was not present in D. excentricus Expt 1.

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Data Files

File	
Density counts of larvae in experiments filename: larvae_density_counts.csv	(Comma Separated Values (.csv), 1.82 KB) MD5:2b0e429c76da06d5a821b9bb3d3f501f
Counts of larvae in count-control beakers used to evaluate the accuracy of larval aliquoting.	
density_morphometrics.csv	(Comma Separated Values (.csv), 67.30 KB) MD5:a97ab7c931eaffbfc3c3ea021b6d6cbb
Primary data file for dataset ID 879120	
Larvae density count file parameters filename: parameters_larvae_density_counts.csv	(Comma Separated Values (.csv), 618 bytes) MD5:d3bf8a85e210f5bc46d0241c2a17be11
Parameter names, descriptions and units of larvae_density_counts.csv.	
Zipped directory of images from which morphometric data was collected filename: larval_images_Peter_Nilsson.zip	(ZIP Archive (ZIP), 385.41 MB) MD5:d24f4b1d3325ac5650222873308e6c38
Microscopic images from four experimental sessions of Dendraster excentricus or Lytechinus pictus larvae.	

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Related Publications

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Usinglme4. Journal of Statistical Software, 67(1). doi:10.18637/jss.v067.i01
Methods

Buitrago, E., Lodeiros, C., Lunar, K., Alvarado, D., Indorf, F., Frontado, K., Moreno, P., & Vasquez, Z. (2005). Mass production of competent larvae of the sea urchin Lytechinus variegatus (Echinodermata: Echinoidea). Aquaculture International, 13(4), 359–367. https://doi.org/10.1007/s10499-004-6551-y
Methods

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous Inference in General Parametric Models. Biometrical Journal, 50(3), 346–363. doi:10.1002/bimj.200810425
Methods

Kacenas, S., & Podolsky, R. (2018). Density-dependent expression of plasticity in larval morphology: effects of actual and apparent competitors. Marine Ecology Progress Series, 593, 1–13. https://doi.org/10.3354/meps12573
Methods

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software, 82(13). doi:10.18637/jss.v082.i13
Methods

Lenth, R., Buerkner, P., Herve, M., Jung, M., Love, J., Miguez, F., Riebl, H., Singmann, H. (2022). emmeans: Estimated Marginal Means, aka Least-Squares Means. Estimated Marginal Means, aka Least-Squares Means. cran.r-project.org. Retrieved from https://cran.r-project.org/web/packages/emmeans/emmeans.pdf
Software

Lowe, C. J., & Wray, G. A. (n.d.). Rearing Larvae of Sea Urchins and Sea Stars for Developmental Studies. Developmental Biology Protocols, 9–15. https://doi.org/10.1385/1-59259-685-1:9
Methods

McAlister, J. S., & Miner, B. G. (Eds.). (2018). Phenotypic Plasticity of Feeding Structures in Marine Invertebrate Larvae. Oxford Scholarship Online. https://doi.org/10.1093/oso/9780198786962.003.0008
Methods

Nilsson, P., & Pernet, B. (2022). Echinoid larvae can express food-conditioned morphological plasticity at ecologically relevant culture densities. Marine Ecology Progress Series, 694, 1–12. https://doi.org/10.3354/meps14111
Results

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. Nature Methods, 9(7), 676–682. doi:10.1038/nmeth.2019
Methods

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Parameters

Parameter	Description	Units
Spawn_date	Date (year-month-day) larvae were spawned.	unitless
Species	Species of larva.	unitless
Experiment	Short name of experiment to which larva belonged.	unitless
Beaker	Name of beaker; unique within experiment but not across experiments.	unitless
Culture_density	Nominal concentration of larvae.	ml-1
Food	Nominal concentration of Rhodomonas lens cells.	ml-1
Right_PO_arm_length	Length of the right postoral arm; measured from tip to the junction of the postoral rod with the transverse rod.	um
Stomach_length	Length of the stomach along the body midline.	um
Midline_body_length	Length of the larva along the body midline.	um
Age	Approximate age at which larvae were photographed.	days
Stage	Stage of larval development; either four-arm (postoral and anterolateral arms only) or six-arm (postoral, anterolateral, and posterodorsal arms).	unitless
Image	Name of image file from which measurements were derived.	unitless

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Instruments

Dataset-specific Instrument Name	QIClick Camera (Teledyne Photometrics)
Generic Instrument Name	Camera
Dataset-specific Description	At set points in the experiments (5 or 7 days), larvae were photographed in dorsal view with a QIClick camera (Teledyne Photometrics) mounted on an Olympus BX-51 compound microscope (Olympus Scientific Solutions) using a 10x (D. excentricus) or 20x (L. pictus) objective. The Fiji distribution of ImageJ (Schindelin et al. 2012) was used to measure the lengths of the right postoral rod, stomach, and body of each photographed larva.
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	BD Accuri C6 Flow Cytometer (BD Biosciences)
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	A BD Accuri C6 Flow Cytometer (BD Biosciences), was used to determine the concentration of Rhodomonas lens.
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	Olympus BX-51 Compound Microscope (Olympus Scientific Solutions)
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	At a set point in the experiments (5 or 7 days) larvae were photographed in dorsal view with a QIClick camera (Teledyne Photometrics) mounted on an Olympus BX-51 compound microscope (Olympus Scientific Solutions) using a 10x (D. excentricus) or 20x (L. pictus) objective.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

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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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