Lipid analysis data from experiment on grazing and physiological effects of ocean acidification on sand dollar larvae (Dendraster excentricus), July 2017

Website: https://www.bco-dmo.org/dataset/753036
Data Type: experimental
Version: 1
Version Date: 2019-01-14

Project
» RUI: Will climate change cause 'lazy larvae'? Effects of climate stressors on larval behavior and dispersal (Climate stressors on larvae)

Contributors	Affiliation	Role
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Abstract
Lipid analysis data collected from a laboratory experiment to investigate the grazing and physiological effects of ocean acidification on sand dollar larvae (Dendraster excentricus), July 2017.

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Coverage

Temporal Extent: 2017-07-07 - 2017-07-31

Dataset Description

Lipid analysis data collected from a laboratory experiment to investigate the grazing and physiological effects of ocean acidification on sand dollar larvae (*Dendraster excentricus*), July 2017.

Methods & Sampling

Spawning and fertilization

We collected adult sand dollars (*D. excentricus*) from Semiahmoo Bay, WA, on July 7, 2017, and maintained them in 14°C continuous flowing seawater at the Shannon Point Marine Center. On July 12, 2017, we induced twelve individuals to spawn by injecting 1-mL of 0.5-M KCl into the coelom following methods outlined by Strathmann (1987). We then collected and mixed concentrated gametes of four males and four females for fertilization. We added five drops of sperm to 500-mL of filtered seawater and 5-mL of eggs. We placed the

fertilized eggs in 12°C incubator and bubbled them with ambient pCO2 condition for 12-hrs before dividing the embryos into pCO2 treatment conditions before gastrulation. We then counted and transferred the larvae into jars with 1.5 L of nanopore filtered seawater at densities of 1-2 individuals mL^{-1}.

Grazing experiment

To assess the interactive effects of high temperature and pCO_2 on *Dunaliella excentricus* feeding behavior, our experimental design had six treatments with four experimental jars (replicates) in each. The treatments combined three levels of CO_2: 400 ppmv (ambient atmospheric level), 800 ppmv (moderate atmoshpheric level) and 1,500 ppmv (high atmospheric level), and two temperatures: 12°C (ambient temperature) and 17°C (high temperature). We fed *D. tertiolecta* at approximately 6,000 cells ml^{-1} to six-arm stage larvae to evaluate feeding rates at each treatment condition.

For each replicate, a corresponding 150-mL control bottle containing only *D. tertiolecta* was also prepared. Feeding rate was estimated as ingestion rate by measuring the algal concentration (cells ml^{-1}) at the beginning (T_0) and after 24 hours (T_f) in control bottles and experimental jars using a Sedgewick Rafter Chamber (Stumpp et al., 2011). Ingestion rate (cells ind^{-1} hr^{-1}) was calculated as I = (Clearance rate) x (time-average algae concentration).

Lipid storage analysis

At the end of the long-term experiment, larval lipid index was assessed using a procedure adapted from Talmage et. al (2010). First, we randomly selected *D. excentricus* larvae from each treatment and stained them with Nile Red dissolved in acetone. Nile Red stains intracellular lipid droplets bright yellow. Larvae were exposed to the stain for ~1.5 h, after which they were photographed under an epi-fluorescent microscope (Leica 80i) within 4 hours of being stained (Ko et al., 2014). The lipid areas of approximately 5−15 larvae per sample were measured using the ImageJ software. The lipid index was calculated by dividing the area of the larva stomach containing the fluorescing lipids by the total stomach area (Talmage & Gobler, 2010).

Data Processing Description

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- reduced precision of lipid_index from 9 to 3 decimals

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Data Files

File	
Lipid_Dendraster_OA_Expt2017.csv	(Comma Separated Values (.csv), 9.82 KB) MD5:46104f166e17be770724e993377f5b5d
Primary data file for dataset ID 753036	

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Related Publications

Ko, G. W. K., Dineshram, R., Campanati, C., Chan, V. B. S., Havenhand, J., & Thiyagarajan, V. (2014). Interactive Effects of Ocean Acidification, Elevated Temperature, and Reduced Salinity on Early-Life Stages of the Pacific Oyster. Environmental Science & Technology, 48(17), 10079–10088. doi:10.1021/es501611u
Methods

Strathmann, M. F. (2017). 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. https://isbnsearch.org/isbn/0-295-96523-1
Methods

Stumpp, M., Dupont, S., Thorndyke, M. C., & Melzner, F. (2011). CO2 induced seawater acidification impacts sea urchin larval development II: Gene expression patterns in pluteus larvae. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 160(3), 320–330. doi:10.1016/j.cbpa.2011.06.023
Methods

Talmage, S. C., & Gobler, C. J. (2010). Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish. Proceedings of the National Academy of Sciences, 107(40), 17246–17251. doi:10.1073/pnas.0913804107
Methods

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Parameters

Parameter	Description	Units
pH_treatment	The pH condition of the water larvae were reared within: L = treatment of 400ppm; M = treatment of 800pm; H = treatment of 1500ppm	unitless
temp_treatment	The temperature condition of the water larvae were reared within	degrees Celsius
jar_replicate	Replicate of the pH and temperature treatment combination. Four jars were maintained at each treatment.	unitless
lipid_presence	Is lipid present within measured larva? Yes or No	unitless
lipid_area	Area of larva's lipid content	pixels per micron
stomach_area	Area of larva's stomach	pixels per micron
lipid_index	Area of larva's lipid content divided by area of larva's stomach	unitless

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Instruments

Dataset-specific Instrument Name	epi-fluorescent microscope (Leica 80i)
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Used to photograph larval intracellular lipid droplets.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

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Project Information

RUI: Will climate change cause 'lazy larvae'? Effects of climate stressors on larval behavior and dispersal (Climate stressors on larvae)

Coverage: Coastal Pacific, USA

In the face of climate change, future distribution of animals will depend not only on whether they adjust to new conditions in their current habitat, but also on whether a species can spread to suitable locations in a changing habitat landscape. In the ocean, where most species have tiny drifting larval stages, dispersal between habitats is impacted by more than just ocean currents alone; the swimming behavior of larvae, the flow environment the larvae encounter, and the length of time the larvae spend in the water column all interact to impact the distance and direction of larval dispersal. The effects of climate change, especially ocean acidification, are already evident in shellfish species along the Pacific coast, where hatchery managers have noticed shellfish cultures with 'lazy larvae syndrome.' Under conditions of increased acidification, these 'lazy larvae' simply stop swimming; yet, larval swimming behavior is rarely incorporated into studies of ocean acidification. Furthermore, how ocean warming interacts with the effects of acidification on larvae and their swimming behaviors remains unexplored; indeed, warming could reverse 'lazy larvae syndrome.' This project uses a combination of manipulative laboratory experiments, computer modeling, and a real case study to examine whether the impacts of ocean warming and acidification on individual larvae may affect the distribution and restoration of populations of native oysters in the Salish Sea. The project will tightly couple research with undergraduate education at Western Washington University, a primarily undergraduate university, by employing student researchers, incorporating materials into undergraduate courses, and pairing marine science student interns with art student interns to develop art projects aimed at communicating the effects of climate change to public audiences

As studies of the effects of climate stress in the marine environment progress, impacts on individual-level performance must be placed in a larger ecological context. While future climate-induced circulation changes certainly will affect larval dispersal, the effects of climate-change stressors on individual larval traits alone may have equally important impacts, significantly altering larval transport and, ultimately, species distribution. This study will experimentally examine the relationship between combined climate stressors (warming and acidification) on planktonic larval duration, morphology, and swimming behavior; create models to generate testable hypotheses about the effects of these factors on larval dispersal that can be applied across systems; and, finally, use a bio-physically coupled larval transport model to examine whether climate-impacted larvae may affect the distribution and restoration of populations of native oysters in the Salish Sea.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1538626

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