

# Effects of food on percent metamorphosis of settling Olympia oysters under different levels of ocean acidification; experiments conducted at Bodega Marine Laboratory, UC Davis in June 2011

**Website:** <https://www.bco-dmo.org/dataset/659651>

**Data Type:** experimental, Other Field Results

**Version:** 19 Sept 2016

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## Project

» [Bodega Ocean Acidification Research](#) (BOAR)

Contributors	Affiliation	Role
<a href="#">Gaylord, Brian</a>	University of California-Davis (UC Davis-BML)	Principal Investigator, Contact
<a href="#">Hill, Tessa M.</a>	University of California-Davis (UC Davis-BML)	Co-Principal Investigator
<a href="#">Russell, Ann D.</a>	University of California-Davis (UC Davis)	Co-Principal Investigator
<a href="#">Sanford, Eric</a>	University of California-Davis (UC Davis-BML)	Co-Principal Investigator
<a href="#">Rauch, Shannon</a>	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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## Dataset Description

Effects of food on percent metamorphosis of settling Olympia oysters under different levels of ocean acidification.

Related datasets:

[larval mass](#)

[larval growth](#)

## Methods & Sampling

### Detailed methodology and results are described in the following publication:

Hettinger, A., E. Sanford, T.M. Hill, J.D. Hosfelt, A.D. Russell, and B. Gaylord. 2013. The influence of food supply on the response of Olympia oyster larvae to ocean acidification. *Biogeosciences* 10: 6629-6638.

doi:[10.5194/bg-10-6629-2013](https://doi.org/10.5194/bg-10-6629-2013)

### Briefly (excerpted from above):

Adult Olympia oysters (n = 140) were collected from Tomales Bay, California (38 deg 06'58" N, 122 deg 51'16" W) in June 2011, transported to Bodega Marine Laboratory (BML) and distributed evenly among four 100 L culturing cylinders. A similar average size of adult oysters was maintained among cylinders. Every day, adults in each cylinder were fed microalgae (*Isochrysis galbana*) at a high concentration to encourage larval release.

Seawater, filtered to 0.45  $\mu\text{m}$  and held at 18– 22 degrees C, was changed every other day. Adults released larvae in two of the four culturing cylinders after 72 h. Larvae were distributed by pipette to 4.5 L glass jars on day 1 of the experiment ( $n = 1000$  larvae per jar). Each jar held 2 L of filtered seawater at the appropriate  $\text{pCO}_2$  level, resulting in initial rearing concentrations of 1 larva per 2 mL of seawater.

In subsequent experimental trials, the investigators employed a target elevated seawater  $\text{pCO}_2$  concentration of 1000  $\mu\text{atm}$  ( $\sim 7.76$  pH). The target control seawater  $\text{pCO}_2$  concentration was 500  $\mu\text{atm}$  ( $\sim 8.05$  pH). All seawater used during larval rearing was pre-adjusted, prior to addition of larvae, to the appropriate  $\text{pCO}_2$  concentrations in 20 L carboys by bubbling filtered seawater for 2–3 days with NIST-traceable  $\text{CO}_2$  air mixtures (carboy water). To minimize net  $\text{CO}_2$  exchange across the seawater surfaces in the jars and help maintain seawater  $\text{pCO}_2$  concentrations at target levels within the jars, the same  $\text{CO}_2$  gas mixtures used to maintain carboy water were pumped continuously into sealed air spaces above the free surfaces of the seawater in the culture jars (hereafter "headspaces"). Five replicate jars per  $\text{pCO}_2$  concentration shared a given headspace. Carboys and jars were held in seawater tables maintained at 20 degrees C ( $\pm 0.2$  degrees C).

Every other day, 90% of the seawater in each jar (jar water) was removed using reverse-filtration through 125  $\mu\text{m}$  mesh and replaced with carboy water at the target  $\text{pCO}_2$  concentration. Immediately following each water change, microalgae (*I. galbana*) were added to each jar to generate final densities in each jar of: 100,000, 50,000, or 10,000 cells per mL ( $n = 2$   $\text{pCO}_2$  levels  $\times 3$  food levels  $\times 5$  replicate jars = 30 jars). The three food levels were the equivalent of initial daily algal cell to larva ratios of 50, 25, and 5 cells per larva per day.

#### *Seawater chemistry:*

Jar water and carboy water were sampled for total alkalinity (TA) and dissolved inorganic carbon (DIC) every other day when a water change was performed. Seawater pH and temperature were measured using a potentiometric pH/temperature meter (Accumet Excel XL60). Raw pH readings (mV) were calibrated using two seawater buffers. pH was monitored primarily as a real-time indicator of changes in the carbonate system. Salinity was determined using a YSI Professional Plus Multiparameter instrument with a conductivity probe (YSI, Yellow Springs, OH). TA was measured using automated Gran titration with duplicates (Metrohm 809), and standardized using certified reference material (CRM) from A. Dickson. The offset between measured and certified TA values for Dickson CRM (Batches 104, 107, 111) was  $-0.01$  ( $\pm 2.81$ ) meq per kg ( $n = 91$ ). Other carbonate system parameters were calculated using the carbonate system analysis software CO2SYS (Lewis and Wallace, 1998).

#### *Sampling of larval growth:*

On day 1, prior to their placement in the culture jars, larvae were collected haphazardly by pipette ( $n = 20$ ), placed on a 125  $\mu\text{m}$  Nitex plankton filter, rinsed, and left to dry at room temperature for 24 h. Larvae were photographed individually under a dissecting microscope (Leica M125 with DC290 camera) for analysis using ImageJ software (ver. 1.37, National Institutes of Health) to determine the initial projected area of the shell. Larvae were sampled similarly on day 5, 9, and 11 post-larval release ( $n = 10$  larvae per jar at each time point). Larval growth was estimated as the change in projected shell area since larval release (day 1).

#### *Sampling of larval total dry weight:*

Total dry weight (body plus shell,  $\mu\text{g}$ ) of the oysters was also determined on days 5, 9, and 11 post larval release. Larvae were collected haphazardly by pipette from each jar ( $n = 25$ ), placed on a 125  $\mu\text{m}$  Nitex plankton filter, and rinsed. Total dry weights of larvae sampled were determined by transferring larvae individually to aluminum vessels pre-ashed at 500 degrees C for 3 h, drying the larval sample at 50 degrees C for  $>24$  h, and weighing on a microbalance (Sartorius Ultramicro, Goettingen, Germany). Larvae were dried in their vessels a second time, reweighed to verify their weights, and combusted at 460 degrees C for 4 h in a muffle furnace (Thermo Scientific FB1415M) to remove all organic matter (see also Gaylord et al., 2011). Ash-free dry tissue weights were determined from the weight difference before and after combusting.

#### *Sampling of percent metamorphosis:*

Settlement of larvae and metamorphosis into benthic juveniles was assessed daily starting on day 11, two days after larval transfers were made into the substrate jars. When  $<5\%$  of larvae remained swimming (used as the assay point for settlement) in each  $\text{pCO}_2$  treatment, the bases of each substrate jar were removed, and the proportion of metamorphosed individuals was determined by first subtracting dead and non-metamorphosed larvae (e.g., pediveligers) from the total initial number of larvae, then dividing by the total initial number of larvae (1000). Any metamorphosed individuals on the walls of the glass jars were also included in the proportion of metamorphosed individuals.

## **Data Processing Description**

### BCO-DMO processing:

- modified parameter names to conform with BCO-DMO naming conventions;
- replaced blanks (missing data) with "nd" ("no data").

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## Data Files

File
<b>food_metamorphosis.csv</b> (Comma Separated Values (.csv), 633 bytes) MD5:1f25efe064ebfdd46b197f5d4701c91e
Primary data file for dataset ID 659651

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## Parameters

Parameter	Description	Units
target_pCO2	Target pCO2	microatmospheres (uatm)
food_level	Food level (low, medium, high)	unitless
headspace	Headspace identifier	unitless
jar	Jar identifier	unitless
num_settlers	Number of settlers	unitless
pcnt_metamorphosis	Percent metamorphosis	percent (%)

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## Instruments

<b>Dataset-specific Instrument Name</b>	Metrohm 809
<b>Generic Instrument Name</b>	Automatic titrator
<b>Dataset-specific Description</b>	TA was measured using automated Gran titration with duplicates (Metrohm 809)
<b>Generic Instrument Description</b>	Instruments that incrementally add quantified aliquots of a reagent to a sample until the end-point of a chemical reaction is reached.

<b>Dataset-specific Instrument Name</b>	DC290
<b>Generic Instrument Name</b>	Camera
<b>Dataset-specific Description</b>	Larvae were photographed individually under a dissecting microscope (Leica M125 with DC290 camera) for analysis using Imagej software.
<b>Generic Instrument Description</b>	All types of photographic equipment including stills, video, film and digital systems.

<b>Dataset-specific Instrument Name</b>	Leica M125
<b>Generic Instrument Name</b>	Microscope - Optical
<b>Dataset-specific Description</b>	Larvae were photographed individually under a dissecting microscope (Leica M125 with DC290 camera) for analysis using Imagej software.
<b>Generic Instrument Description</b>	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".

<b>Dataset-specific Instrument Name</b>	YSI Professional Plus Multiparameter instrument
<b>Generic Instrument Name</b>	Multi Parameter Portable Meter
<b>Dataset-specific Description</b>	Salinity was determined using a YSI Professional Plus Multiparameter instrument with a conductivity probe (YSI, Yellow Springs, OH).
<b>Generic Instrument Description</b>	An analytical instrument that can measure multiple parameters, such as pH, EC, TDS, DO and temperature with one device and is portable or hand-held.

<b>Dataset-specific Instrument Name</b>	Accumet Excel XL60
<b>Generic Instrument Name</b>	pH Sensor
<b>Dataset-specific Description</b>	Seawater pH and temperature were measured using a potentiometric pH/temperature meter (Accumet Excel XL60).
<b>Generic Instrument Description</b>	An instrument that measures the hydrogen ion activity in solutions. The overall concentration of hydrogen ions is inversely related to its pH. The pH scale ranges from 0 to 14 and indicates whether acidic (more H+) or basic (less H+).

<b>Dataset-specific Instrument Name</b>	Accumet Excel XL60
<b>Generic Instrument Name</b>	Water Temperature Sensor
<b>Dataset-specific Description</b>	Seawater pH and temperature were measured using a potentiometric pH/temperature meter (Accumet Excel XL60).
<b>Generic Instrument Description</b>	General term for an instrument that measures the temperature of the water with which it is in contact (thermometer).

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## Deployments

### BML\_Gaylord

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/658395">https://www.bco-dmo.org/deployment/658395</a>
<b>Platform</b>	lab Bodega Marine Laboratory
<b>Start Date</b>	2010-09-01
<b>End Date</b>	2011-06-30

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## Project Information

### Bodega Ocean Acidification Research (BOAR)

**Website:** <http://bml.ucdavis.edu/research/research-programs/climate-change/oceanacidification/>

**Coverage:** Central California coast (northeast Pacific)

The absorption of human-produced CO<sub>2</sub> into the world's oceans is decreasing seawater pH and causing marked declines in the saturation state for calcium carbonate, a major building block for shells, skeletons, and tests of many marine species. Such changes (collectively termed "ocean acidification") have the potential to devastate a broad array of organisms, both at the level of individuals and at population and ecosystem scales. Although awareness of these issues is rapidly growing, most of what is known is based on studies of coral reef organisms and plankton.

The proposed work will enhance understanding of impacts from ocean acidification by providing rigorous data on several new fronts applicable to temperate systems. The project will operate within one of the strongest upwelling centers of the eastern Pacific, where global trends in acidification are amplified by the presence of cold water characterized by already-high levels of aqueous CO<sub>2</sub>. Using an integrated, comparative approach that exploits the expertise of oceanographers, marine chemists, and biologists, the project will explicitly couple moored and shipboard measurements of seawater chemistry to controlled laboratory and field studies of biological responses.

Two vital foundation species (the California mussel, *Mytilus californianus*, and the Olympia oyster, *Ostrea conchaphila*) will be targeted. These two species play disproportionately important roles in open-coast and estuarine systems, respectively. Larvae (which are often the most vulnerable stages) of mussels and oysters will be cultured under elevated-CO<sub>2</sub> conditions through the full pelagic period and into juvenile life. Growth and survivorship will be quantified, and water temperature and salinity will be varied to test for interactive effects of multiple factors. Intraspecific variation in response of larvae from different parental lineages will be examined. "Carry-over" effects that originate from exposure during the larval stage, but influence subsequent juvenile growth and survival, will be determined both in the laboratory and using field outplants. Because larval and juvenile stages play important roles as demographic age-structure bottlenecks, overall population

consequences will be estimated through comparison of observed impacts on early life stages to other recognized sources of recruitment variation.

**Data Status:** Data will be reported from the BML offshore oceanographic moorings and from moorings within nearby Tomales Bay. The moorings will be outfitted with autonomously recording pH and pCO<sub>2</sub> sensors, and these measurements will be supplemented with discrete water samples collected monthly along two associated transects.

**Live Data:** For live-streaming data from Tomales Bay, visit <http://www.ipacoa.org/Explorer> and click on the icon in Tomales Bay.

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-0927255</a>

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