

Juvenile growth post-settlement under four larval-to-juvenile ocean acidification transitions in the laboratory; conducted at Bodega Marine Lab in July-Sept 2009

Website: <https://www.bco-dmo.org/dataset/658894>

Data Type: experimental, Other Field Results

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Project

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

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Dataset Description

Juvenile growth (on day 7 post-settlement) of Olympia oysters under four larval-to-juvenile ocean acidification transitions in the laboratory. The study examined the consequences of decreased pH and carbonate saturation on the early life stages of Olympia oysters. Oysters were reared in three levels of seawater pH (8.0, 7.9, 7.8) and their growth rates were measured/compared.

Related datasets:

[juvenile growth - experiment 1](#)

[larval growth - experiment 1](#)

Methods & Sampling

Detailed methodology and results are described in following publication:

Hettinger, A., E. Sanford, T.M. Hill, A.D. Russell, K.N. Sato, J. Hoey, M. Forsch, H.N. Page, and B. Gaylord. 2012. Persistent carry-over effects of planktonic exposure to ocean acidification in the Olympia oyster. Ecology 93: 2758-2768. doi:[10.1890/12-0567.1](https://doi.org/10.1890/12-0567.1)

Briefly (excerpted from above):

Oysters were reared at Bodega Marine Laboratory (BML) in two experiments. These data are from experiment 1. In both, seawater CO₂ concentrations in the treatment cultures were increased relative to present-day levels by 100 and 400 ppm. Thus, seawater CO₂ concentrations employed in the experiments were: 700 (used as an operational control), 800, and 1100 ppm. Such CO₂ levels correspond to pH values of approximately 8.0, 7.9, and 7.8 (NBS scale). These nominal pH levels were used subsequently to identify the treatments.

Oysters were reared from early larval life in 4.5-L glass culture jars held in seawater tables maintained at 20.0 (+/- 0.02) degrees C. All seawater used during rearing was filtered at 0.45 µm and pre-adjusted to appropriate pH levels in 20 L carboys by bubbling for 2-3 days with NIST-traceable CO₂ air mixtures (hereafter referred to as "carboy water"). Acrylic boxes mounted over each seawater table received the same mixed gases and provided a common head space for six jars, minimizing off-gassing during culturing. Levels of pH used for each box, and jar position within a box, were randomly assigned.

Larval culturing:

Adult Olympia oysters (4-7 cm in length) were collected from Tomales Bay and transported to BML. They were cleaned and distributed among multiple 100-L cylinders containing seawater filtered at 0.45 µm and held at 18-22 degrees C. At least one female per cylinder released larvae within 48 hours post-collection, enabling acquisition of independent "larval cohorts". Following release, larvae were transferred into culture jars containing 2L of seawater filtered at 0.45 µm (day 1 of the experiment). Every other day, 90% of the seawater in each jar was changed and replaced with carboy water, whose pH had stabilized to the appropriate level.

Water chemistry:

Samples of jar water and carboy water were collected every day. Seawater pH (NBS) and temperature were measured using a pH/temperature meter (Accumet Excel XL60; Thermo Fisher Scientific, Waltham, Massachusetts, USA), and salinity was determined using a YSI 6600V2 multiparameter instrument (YSI, Yellow Springs, Ohio, USA). Alkalinity was measured using automated Gran titration (Metrohm 809; Metrohm, Herisau, Switzerland), and standardized using certified reference material from A. Dickson at Scripps Institution of Oceanography. Other carbonate system parameters were calculated using the software, CO₂SYS (Lewis and Wallace 1998).

Sampling of larvae and juveniles:

Oysters in the culture jars were sampled at key time points during each experiment to quantify shell size and growth rate (change in shell area per day). On day 1, 100 larvae per larval cohort were collected haphazardly by pipette, fixed in 95% ethanol, and individually photographed under a microscope (Leica DM1000 with DC290 camera; Leica Microsystems, Wetzlar, Germany) for analysis using ImageJ software (version 1.37) to determine the initial projected area of the shell (software available online). Larval shell growth rates at later time points were calculated similarly. Juvenile shell growth rate following settlement was determined by measuring the projected shell area of settled individuals from photographs, subtracting the area of the larval shell (which remains visually distinct), and dividing that value by the number of days postsettlement.

Experiment 2:

Reduced pH induced a negative effect on juvenile growth rate. This outcome (of experiment 1) could have arisen from one of two causes: direct effects of seawater acidification during the early juvenile phase or carry-over effects of larval experience. Experiment 2 (September–October 2009) was conducted to distinguish among these alternatives.

In the first phase of Experiment 2, larvae were pooled across cohorts (300 larvae/cohort; 900 larvae/replicate jar) and reared through settlement under either control or low pH conditions (pH 8.0 or 7.8). The pH levels were randomly assigned to four boxes (2 pH levels x 2 boxes x 3 replicate jars = 12 jars). In the second phase of the experiment, and within 24 h of larval settlement, one-half of the juveniles reared as larvae in control pH conditions were transferred to low pH conditions, and one-half were returned to control pH conditions. Similarly, one-half of the juveniles that had been reared as larvae in low pH conditions were transferred to control pH conditions, and one-half were returned to low pH conditions.

The second phase of the experiment used 2 pH levels x 4 larval-juvenile pH treatments x 6 replicate jars = 24 jars. The juveniles in the two destination pH levels were reared until 13 days after settlement, and randomly selected juveniles on wedges (N=17 per jar) were photographed 7 and 13 days after settlement to quantify shell growth rate since settlement. Larval settlement occurred 24 hours earlier (day 13) under low pH conditions; therefore all photographs taken of settled juveniles in control conditions were delayed by one day.

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
juv_growth_exp2.csv (Comma Separated Values (.csv), 904 bytes) MD5:9900aa603bc015932512c3753e1037b6
Primary data file for dataset ID 658894

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Parameters

Parameter	Description	Units
pH_nominal_larval	Nominal pH level during larval phase	pH scale (NBS)
pH_nominal_juv	Nominal pH level during juvenile phase	pH scale (NBS)
avg_size_at_settlement	Average size at settlement	square millimeters (mm ²)
avg_juv_growth	Average juvenile growth	square millimeters (mm ²)
avg_juv_growth_rate	Average juvenile growth rate	square millimeters per day (mm ² /day)

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Instruments

Dataset-specific Instrument Name	DC290
Generic Instrument Name	Camera
Dataset-specific Description	Individuals were examined under a microscope (Leica DM1000 with DC290 camera)
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Leica DM1000
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Individuals were examined under a microscope (Leica DM1000 with DC290 camera)
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".

Dataset-specific Instrument Name	YSI 6600V2
Generic Instrument Name	Multi Parameter Portable Meter
Dataset-specific Description	Salinity was determined using a YSI 6600V2 multi-parameter instrument (YSI, Yellow Springs, Ohio)
Generic Instrument Description	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.

Dataset-specific Instrument Name	Accumet Excel XL60
Generic Instrument Name	pH Sensor
Dataset-specific Description	Seawater pH (NBS) and temperature were quantified using a pH/temperature meter: Accumet Excel XL60 (Thermo Fisher Scientific, Waltham, MA)
Generic Instrument Description	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 ⁺).

Dataset-specific Instrument Name	Metrohm 809
Generic Instrument Name	Titration
Dataset-specific Description	Alkalinity was measured using automated Gran titration (Metrohm 809; Metrohm, Herisau, Switzerland)
Generic Instrument Description	Titration is an instrument that incrementally adds quantified aliquots of a reagent to a sample until the end-point of a chemical reaction is reached.

Dataset-specific Instrument Name	Accumet Excel XL60
Generic Instrument Name	Water Temperature Sensor
Dataset-specific Description	Seawater pH (NBS) and temperature were quantified using a pH/temperature meter: Accumet Excel XL60 (Thermo Fisher Scientific, Waltham, MA)
Generic Instrument Description	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

Website	https://www.bco-dmo.org/deployment/658395
Platform	lab Bodega Marine Laboratory
Start Date	2010-09-01
End Date	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 CO₂ 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 CO₂. 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-CO₂ 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 pCO₂ 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
NSF Division of Ocean Sciences (NSF OCE)	OCE-0927255

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