Carbonate chemistry, shell growth, and respiration data from laboratory experiments on California mussel larvae condcuted at the Hatfield Marine Science Center, Newport, OR in 2013

Website: https://www.bco-dmo.org/dataset/557253
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Project
» A mechanistic understanding of the impacts of ocean acidification on the early life stages of marine bivalves (Mechanisms of bivalve response to acidification)

Program
» Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Contributors	Affiliation	Role
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Table of Contents

Dataset Description

Carbonate chemistry, shell growth, and respiration data from laboratory experiments on California mussel larvae (*Mytilus californianus*). Experiments were conducted in the Hatfield Marine Science Center, Newport, OR.

Related publications:
Waldbusser et al. 2015 (*in press*). Ocean acidification has multiple modes of action on bivalve larvae. PLOSOne.

Methods & Sampling

Methods below from Waldbusser et al. 2015 (*in press*). Ocean acidification has multiple modes of action on bivalve larvae. PLOSOne.

Carbonate chemistry measurements
Discrete samples were taken for carbonate chemistry at three time points: 1) following decarbonation but prior to manipulation, 2) immediately prior to filling BOD bottles from gas-impermeable bags, and 3) from each BOD bottle at the termination of the development experiment. Treatment data presented are from the samples in step #2 above. Seawater samples at the termination of the experiment were taken by placing a siphon tube into the BOD bottles, with its submerged end covered with a 37 micron Nitex screen to prevent removal of larvae. The siphon was started and the initial seawater flow discarded before sample collection with minimal aeration in a 350 ml amber glass bottle. All seawater samples collected in 350 ml amber glass bottles were preserved with 30 ml of saturated HgCl2, and sealed with polyurethane-lined metal crimp caps. PCO2 and DIC analyses were carried out via gas equilibration and stripping, respectively, followed by infrared detection, as in

Bandstra et al. 2006 and Hales et al. 2005, modified for discrete samples. Standards for PCO2 and DIC encompassed the complete range of values in this study which are outside the range of typical modern-ocean seawater. To compute the complete carbonate chemistry we used, Millero 2010 carbonic acid dissociation constants with temperature and salinity dependencies (which capture the Lueker et al. 2000 seawater constants), Dickson 1990 constants for boric acid, and Millero 1995 water dissociation constants.

Proportion Normal
At termination of the development experiment (48 hours post-fertilization) and after seawater samples had been taken, larvae were concentrated in a known volume of seawater and three replicate samples were collected from each BOD bottle and stored in 20 ml vials and preserved with buffered (pH 8.1-8.2) formalin. The average number of total larvae per replicate vial from each BOD bottle was 79 (±20), with a range from ~ 50 to ~100.

The proportion of normally developed larvae 48 hours post fertilization was evaluated under an inverted microscope, with normal development noted as a completely formed D-shaped shell with a straight hinge and the presence of tissue inside the shell. We previously found that velum extrusion was sometimes related to the speed and strength of additions of buffered formalin; therefore, we scored larvae as normal if larvae showed minor velum extrusion and normally developed shells.

Shell Growth
Shell size was determined by taking photographs of all sampled larvae scored as normally developed using an inverted transmission microscope (Jena Sedival 250-CL coupled to a FujiFilm Digital SLR S5) at 50x magnification and measuring shell lengths (longest axis parallel to shell hinge) on size calibrated images (ImageJ v 1.42). Shell lengths were measured for a total of 4639 normally developed individual larvae. We only measured size of normally developed larvae to prevent bias in the size estimates (poorly or undeveloped larvae are always smaller) and to prevent conflation of developmental and growth effects in interpreting results.

Respiration Rate
Respiration rates were measured for 48-h larvae that were first pooled from triplicate BOD bottles of each seawater treatment, after samples had been taken from each BOD bottle for determination of development and water chemistry analysis. Larvae were stocked at an estimated concentration of 500 larvae in each of five replicate 2 ml, solid-capped GC vials filled with the same seawater treatment as they had experienced during development in the BOD bottles. Larvae were concentrated and enumerated prior to stocking such that the larvae suspension constituted less than 10% of the volume added to each 2 ml vial. The desired concentration of 500 larvae per vial was chosen to yield the strongest and most consistent respiration signal while minimizing the effect of respiration on ambient carbonate chemistry within each treatment. Preliminary analyses showed that respiration rates under normal conditions were unaffected by larval densities between 200 and 800 larvae per vial (data not shown). Fluorescent oxygen-sensitive sensor spots (5 mm planar oxygen-sensitive spots, PSt3, PreSens, Germany) were pre-attached to the base of each vial before seawater or larvae were added. Oxygen measurements were made using the Fibox 3 (PreSens, Germany) that utilizes a fiber-optic cable to transmit and receive light from a sensor spot through the glass vial, allowing oxygen measurements to be taken in a non-destructive manner and without opening the vials. The vials were filled with warmed (18 degrees C) seawater siphoned from seawater contained in the same impermeable bags as that used to fill the BOD bottles, followed with additions of chloramphenicol and 10 ppm ampicillin to control bacterial respiration. Five control vials (without additions of larvae) per seawater treatment allowed correction of larval respiration measurements for background bacterial respiration. Larval respiration rates were approximately 2 – 3 times those of background bacterial rates. Following larval additions, solid caps with PTFE liners were tightly screwed onto each vial, taking care to eliminate any bubbles from the vials. Vials were held on their sides submerged in a temperature-controlled seawater bath to maintain an experimental temperature of 18 degrees C. Oxygen measurements on all 60 vials were taken every 2 hours over the first 6 hours of incubation (including time 0, initiation of respiration measurements), allowing linear regression of four time-points per treatment. We used a 6-hour incubation period because longer periods resulted in reduced respiration rates. The slopes of the regressions were corrected for background bacterial respiration determined in the control vials. Once respiration rate measurements were completed, larvae from each vial were preserved, counted and respiration rates expressed per larva (abnormal plus normal). The total average number of larvae per vial was 457 +/- 89 (1 standard deviation).

Initiation of Feeding
To assess the impact of water treatments on development of feeding organs and processes, we determined the proportion of larvae that ingested fluorescent beads at 44 hours post fertilization (initiation of feeding; IF). Preliminary experiments showed that at 44 hours post-fertilization ≥50% of *M. californianus* larvae began feeding when reared in natural seawater at 18 degrees C. Fertilized eggs were stocked at 10 ml-1 in triplicate 20 ml solid-capped, sealed vials completely filled (no head space) with seawater treatments, at the same time

as larvae were stocked in BOD bottles. After addition of fertilized eggs, 2µm Fluorescbrite® yellow beads (excitation maxima at 441nm and emission maxima at 485nm; Polysciences Inc., Warrington, PA) were added to the vials at a concentration of 20 beads ul-1. Larval density effects, including the density used here, on microsphere availability were found to be negligible during preliminary experiments. Although these are higher than found in natural systems, for short-term, experimental comparisons our larval and bead densities did not appear to skew results. At 44 hour post-fertilization, the experiment was terminated by adding 40 ul of 10% buffered formalin (pH ~8.1-8.2) to the vials. A minimum of 20 larvae per replicate vial were later examined under epifluorescent microscopy for the presence or absence of beads within their guts. The proportion of larvae feeding was then determined as the ratio of larvae that had at least 1 bead in their gut to the total # of larvae counted.

References

Bandstra L, Hales B and Takahashi T. High-frequency measurements of total CO2: method development and first oceanographic observations. Mar Chem. 2006;100: 24-38.

Hales B, Chipman D, and Takahashi T. High-frequency measurement of partial pressure and total concentration of carbon dioxide in seawater using microporous hydrophobic membrane contactors. Limnol Oceanogr Methods. 2005;2: 356-364.

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Lueker TJ, Dickson AG and Keeling CD. Ocean pCO2 calculated from dissolved inorganic carbon, alkalinity, and equations for K-1 and K-2: validation based on laboratory measurements of CO2 in gas and seawater at equilibrium. Mar Chem. 2000;70: 105-119.

Dickson AG. Standard potential of the reaction - Agcl(S)+1/2h-2(G)=Ag(S)+Hcl(Aq) and the standard acidity constant of the ion Hso4- in synthetic sea-water from 273.15-K to 318.15-K. J Chem Thermodyn. 1990;22: 113-127.

Millero FJ. Thermodynamics of the carbon-dioxide system in the oceans. Geochim Cosmochim Ac. 1995;59: 661-677.

Data Processing Description

BCO-DMO Processing:
- Replaced blanks (missing data/not measured) with 'nd' to indicate 'no data'.
- Modified parameter names to conform with BCO-DMO naming conventions.
- Added taxon column.

[table of contents | back to top]

Data Files

File	
cal_mussels.csv	(Comma Separated Values (.csv), 1.82 KB) MD5:78a3f6dd93d08ed78892e39abb6bd0f8
Primary data file for dataset ID 557253	

[table of contents | back to top]

Parameters

Parameter	Description	Units
taxon	Name of species studied.	dimensionless (text)
omega_trtmnt	Saturation state (omega Aragonite) treatment condition. Four treatment levels were used encompassing a range from ~0.5 to 5.0; targets were 0.50, 1.0, 2.0, 4.0. Treatment values for saturation state of aragonite are noted as "a" to "d" denoting the lowest to highest values.	dimensionless (text)
PCO2_trtmnt	Partial pressure of carbon dioxide (PCO2) treatment condition. Four treatment levels were used encompassing a range from ~200 to 2500 microatmospheres (uatm); targets were 200, 400, 800, 2500. Treatment values for PCO2 are noted as Low (L), Mid-Low (ML), Mid_High (MH), and High (H).	dimensionless (text)
total_alk	Total alkalinity.	micromoles per kilogram (umol/kg)
DIC	Dissolved inorganic carbon (DIC).	micromoles per kilogram (umol/kg)
PCO2	Partial pressure of carbon dioxide (PCO2).	micromoles per kilogram (umol/kg)
HCO3	Bicarbonate ion (HCO3).	micromoles per kilogram (umol/kg)
CO3	Carbonate ion (CO3).	micromoles per kilogram (umol/kg)
pH	pH.	pH scale.
omega_A	The saturation state of seawater with respect to aragonite.	dimensionless
prop_norm	The proportion of normally developed larvae 48 hours post fertilization.	dimensionless (proportion)
prop_norm_stdev	Standard deviation of the proportion of normally developed larvae.	dimensionless (proportion)
shell_length	Shell length (longest axis parallel to shell hinge) as measured on size calibrated images.	micrometers (um)
shell_length_stdev	Standard deviation of shell length.	micrometers (um)
respir_rate	Respiration rate (see acquisition description for methods). Once respiration rate measurements were completed, larvae from each vial were preserved, counted and respiration rates expressed per larva (abnormal plus normal).	picomoles O2 per hour per individual (pmol O2 /hr ind)
respir_rate_stdev	Standard deviation of respiration rate.	picomoles O2 per hour per individual (pmol O2 /hr ind)
prop_feeding	The proportion of larvae feeding determined as the ratio of larvae that had at least 1 bead in their gut to the total # of larvae counted (see acquisition description for methods).	dimensionless (proportion)
prop_feeding_stdev	Standard deviation of the proportion of larvae feeding.	dimensionless (proportion)

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	biological oxygen demand (BOD) bottles
Generic Instrument Name	Bottle
Generic Instrument Description	A container, typically made of glass or plastic and with a narrow neck, used for storing drinks or other liquids.

Dataset-specific Instrument Name	epifluorescent microscope
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Epifluorescent microscopy was used to determine the proportion of larvae feeding.
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.

Dataset-specific Instrument Name	non-dispersive infra-red (NDIR) gas analyzer
Generic Instrument Name	Gas Analyzer
Dataset-specific Description	PCO2 and DIC analyses were carried out via gas equilibration and stripping, respectively, followed by infrared detection, as in Bandstra et al. (Reference: Bandstra L, Hales B and Takahashi T. High-frequency measurements of total CO2: method development and first oceanographic observations. Mar Chem. 2006;100: 24-38. doi:10.1016/j.marchem.2005.10.009)
Generic Instrument Description	Gas Analyzers - Instruments for determining the qualitative and quantitative composition of gas mixtures.

Dataset-specific Instrument Name	Inverted transmission microscope
Generic Instrument Name	Inverted Microscope
Dataset-specific Description	Shell size was determined by taking photographs of all sampled larvae scored as normally developed using an inverted transmission microscope (Jena Sedival 250-CL coupled to a FujiFilm Digital SLR S5) at 50x magnification and measuring shell lengths (longest axis parallel to shell hinge) on size calibrated images (ImageJ v 1.42).
Generic Instrument Description	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

[table of contents | back to top]

Deployments

Waldbusser_HMSC

Website	https://www.bco-dmo.org/deployment/557259
Platform	OSU-HMSC
Start Date	2013-08-19
Description	Laboratory experiments on California mussel larvae (Mytilus californianus) were conducted in the Hatfield Marine Science Center, Newport, OR.

[table of contents | back to top]

Project Information

A mechanistic understanding of the impacts of ocean acidification on the early life stages of marine bivalves (Mechanisms of bivalve response to acidification)

Coverage: Coastal and estuarine waters of Oregon, U.S.A.

Extracted from the NSF award abstract:

The shift in the carbonate chemistry of marine waters, as a result of direct anthropogenic CO2 addition and climate-driven changes in circulation, poses a threat to many organisms. A rapidly expanding body of literature has shown that increasing levels of carbonic acid and decreasing carbonate ion levels will have deleterious

effects on many marine organisms; however little is known about the mode of action of these changes in water chemistry on marine bivalves. Many marine organisms, particularly bivalves, depend critically on the production of calcium carbonate mineral, and this material becomes thermodynamically unstable under more acidic conditions. The actual mineral precipitation, however, takes place within interstitial volumes intermittently separated from ambient seawater by biological membranes. Therefore, abiotic relationships between solid phase minerals and seawater thermodynamics are oversimplified representations of the complex interplay among seawater chemistry, bivalve physiology, and shell growth processes.

In this integrative, multi-disciplinary project we will develop and apply novel experimental approaches to elucidate fundamental physiological responses to changes in seawater chemistry associated with ocean acidification. The four primary objectives of this project are to: 1) develop a novel experimental approach and system capable of unique combinations of pCO2, pH, and mineral saturation state (Ω), 2) conduct short-term exploratory experiments to determine bivalve responses to different carbonate system variables, 3) conduct longer-term directed studies of the integrated effects of different carbonate system variables over early life history of bivalves, and 4) compare these biological responses among a group of bivalve species that differ in shell mineralogy and nativity to the periodically acidified upwelling region of the Pacific Northwest coast of North America. By isolating the effects of different components of the carbonate system on the early life stages of marine bivalves, e.g. does an oyster larvae respond more strongly to pCO2 or mineral saturation state?, we can begin to identify the mechanisms behind bivalve responses as well as understand how these organisms survive in transiently corrosive conditions.

Laboratory based experiments on three primary taxa (oyster, mussel, clam) having native and non-native species pairs to Oregon's coastal waters: oysters *Ostrea lurida* and *Crassostrea gigas*; mussels *Mytilus califonianus* and *Mytilus galloprovincialis*; and clams *Macoma nasuta* and *Ruditapes philippinarum*, will allow for species comparisons among different shell mineralogy, microstructure, life-history, and adaptability. High-precision pCO2 and dissolved inorganic carbon (DIC) instruments will be used in experiments to control and properly constrain the carbonate chemistry. A compliment of response variables will be measured across the early life stages of these species that include tissue acid-base balance, shell mineralogy and chemistry, respiration rate, and behavior. Additionally, our emphasis will be placed on observation of development, growth, and shell structure by directly linking observational data with other measured response data. An adaptive strategy using short-term experiments to determine the most salient variables in the carbonate system to manipulate in longer-term studies is being employed. This approach allows us to evaluate acute effects, mimicking diurnal changes to carbonate variables often found in coastal areas, and integrated chronic effects mimicking a more gradual acidification due to the rise in atmospheric CO2.

[table of contents | back to top]

Program Information

Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Website: https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503477

Coverage: global

NSF Climate Research Investment (CRI) activities that were initiated in 2010 are now included under Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES). SEES is a portfolio of activities that highlights NSF's unique role in helping society address the challenge(s) of achieving sustainability. Detailed information about the SEES program is available from NSF (https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=504707).

In recognition of the need for basic research concerning the nature, extent and impact of ocean acidification on oceanic environments in the past, present and future, the goal of the SEES: OA program is to understand (a) the chemistry and physical chemistry of ocean acidification; (b) how ocean acidification interacts with processes at the organismal level; and (c) how the earth system history informs our understanding of the effects of ocean acidification on the present day and future ocean.

Solicitations issued under this program:

NSF 10-530, FY 2010-FY2011
NSF 12-500, FY 2012
NSF 12-600, FY 2013
NSF 13-586, FY 2014
NSF 13-586 was the final solicitation that will be released for this program.

PI Meetings:
1st U.S. Ocean Acidification PI Meeting(March 22-24, 2011, Woods Hole, MA)
2nd U.S. Ocean Acidification PI Meeting(Sept. 18-20, 2013, Washington, DC)
3rd U.S. Ocean Acidification PI Meeting (June 9-11, 2015, Woods Hole, MA – Tentative)

NSF media releases for the Ocean Acidification Program:

Press Release 10-186 NSF Awards Grants to Study Effects of Ocean Acidification

Discovery Blue Mussels "Hang On" Along Rocky Shores: For How Long?

Discovery nsf.gov - National Science Foundation (NSF) Discoveries - Trouble in Paradise: Ocean Acidification This Way Comes - US National Science Foundation (NSF)

Press Release 12-179 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: Finding New Answers Through National Science Foundation Research Grants - US National Science Foundation (NSF)

Press Release 13-102 World Oceans Month Brings Mixed News for Oysters

Press Release 13-108 nsf.gov - National Science Foundation (NSF) News - Natural Underwater Springs Show How Coral Reefs Respond to Ocean Acidification - US National Science Foundation (NSF)

Press Release 13-148 Ocean acidification: Making new discoveries through National Science Foundation research grants

Press Release 13-148 - Video nsf.gov - News - Video - NSF Ocean Sciences Division Director David Conover answers questions about ocean acidification. - US National Science Foundation (NSF)

Press Release 14-010 nsf.gov - National Science Foundation (NSF) News - Palau's coral reefs surprisingly resistant to ocean acidification - US National Science Foundation (NSF)

Press Release 14-116 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: NSF awards $11.4 million in new grants to study effects on marine ecosystems - US National Science Foundation (NSF)

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1041267

[table of contents | back to top]