Chemistry and organism response data from Waldbusser et al. 2015, Nature Climate Change; from experiments conducted at the Hatfield Marine Science Center, Newport, OR in 2013

Website: https://www.bco-dmo.org/dataset/638362
Data Type: experimental
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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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Dataset Description

Chemistry and organism response data from Waldbusser et al. 2015, Nature Climate Change. Experiments were conducted in the Hatfield Marine Science Center, Newport, OR.

Related publications:
Waldbusser, G., et al. 2015. Saturation-state sensitivity of marine bivalve larvae to ocean acidification. Nature Climate Change, 5, 273-280. doi:10.1038/nclimate2479

Methods & Sampling

Methods (see SI in Waldbusser et al. 2015, Nature Climate Change for extended methods):
Water collection and stripping dissolved inorganic carbon:
For each experiment, 1 um filtered seawater was collected from Yaquina Bay, Oregon. The alkalinity was reduced by addition of trace metal grade HCl in near alkalinity equivalence, followed by bubbling with ambient air for 48 hours to strip (DIC) as CO2. The acidified, stripped seawater was then 0.22 um filtered, pasteurized, and stored at 2-5 degrees C. Prior to treatment manipulation, the seawater was bubbled with 0.2 um-filtered outside air until atmospheric conditions were achieved, then carbonate DIC and alkalinity values were determined for manipulations.

Experimental manipulation:
A 4x4 factorial experimental design was developed to target 16 total treatment combinations of PCO2 and War

(saturation state with respect to aragonite) (Supplementary Table 1, Figure 1), with triplicate 500 ml biological oxygen demand (BOD) bottles per treatment. Two separate experiments were conducted with each species. DIC and alkalinity concentrations were calculated for each of the 16 target treatment combinations (PCO2 and War). Experimental treatments were created by gravimetric addition of mineral acids and bases to the decarbonated seawater in gas-impermeable bags customized with luer lock fittings. Aliquots of a concentrated, ambient-PCO2, solution of Na2CO3 and and NaHCO3 were added to adjust DIC to target treatment level followed by 0.1N HCl to adjust alkalinity. Immediately following chemical manipulation, the bags with treatment water were stored without head-space at 2-5 degrees C for up to several weeks before spawning broodstock. Antibiotics were added to BOD bottles (2 ppm chloramphenicol and 10 ppm ampicillin), which we found to have no negative effects on larvae or carbonate chemistry in prior trials. Controls were included to evaluate experimental manipulations and incubation conditions by hatching eggs in open culture containers, as well as by using stored seawater collected prior to decarbonation and not subjected to chemical manipulations described in this study.

Carbonate chemistry measurements:
Carbonate chemistry samples were collected from the treatment water bags just prior to stocking larvae in BOD bottles, and also from each BOD bottle at the end of the incubation period. Carbonate chemistry samples were collected in 350 ml amber glass bottles with polyurethane-lined crimp-sealed metal caps and preserved by addition of 30 ml of saturated HgCl2. Analyses of PCO2 and DIC were carried out following the procedure of Bandstra et al. modified for discrete samples as in Hales et al. Gas and liquid standards that bracketed the experimental range (Supplementary Table 1) were employed to ensure accuracy.

Larval Rearing:
Broodstock for mussel (*Mytilus galloprovincialis*) and oyster (*Crassostrea gigas*) experiments were obtained from Carlsbad Aquafarm, Carlsbad, CA, or from selected stocks of the Molluscan Broodstock Program (MBP), Yaquina Bay, Oregon, respectively. Broodstock spawning was stimulated by rapid increase of 10 degrees C in ambient seawater temperature. Gametes were collected from at least two male and two female parents, and the eggs fertilized in ambient seawater. Developing embryos were added at a density of 10 larvae ml-1 to triplicate BOD bottles per treatment after visual verification of successful fertilization. Sealed BOD bottles were oriented on their side and incubated for 48 hours at culture temperature (18 degrees C for mussels and 22 degrees C and 25 degrees C for oyster trials 1 and 2, respectively). Larvae from each BOD bottle were concentrated after a filtered chemistry sample was collected, sampled in triplicate, and preserved in 10% formalin buffered to ~8.1-8.2.

Larval shell development and size:
Larvae were examined microscopically to determine the proportion of normally and abnormally developed D-hinge (prodissoconch I) larvae as well as larval shell lengths. Normally developed larvae were characterized by a straight hinge, smooth curvature along the edge of the valve, and appearance of tissue within the translucent shells. Digital images were used to determined shell length (longest axis perpendicular to the hinge) of normally developed larvae only. Images were analyzed using ImageJ (V1.42).

Data analyses:
Proportion normal data were scaled to the un-manipulated, seawater control for each experiment by dividing treatment values by control values. We used a two-way analysis of variance (ANOVA), with PCO2 and War as the primary factors with experiment as a blocking factor. Proportion normal data were square-root arcsine transformed. Assumptions of normality and homoscedascity were checked and any violations were managed as noted. Initial data analyses found unequal variance across treatment groups in the transformed proportion normal data, and mean values per treatment were used to improve heteroscedascity as well as blocking by experiment. To evaluate pH effects on shell development we ran a series of regression analyses of transformed proportion normal regressed on pH, within each War treatment and experiment. We then used a Bonferroni correction for multiple tests of significance to reduce Type 1 error. Analyses were conducted with the SAS software suite (v9.3). Non-linear, least-squares regression in Sigma-Plot (v12.5) was used to fit functional responses of development (logistic) and shell length (power).

References:
Bandstra L, Hales B & Takahashi T. 2006. High-frequency measurements of total CO2: Method development and first oceanographic observations. *Mar Chem* 100(1-2)**:** 24-38.

Hales B, Takahashi T & Bandstra L. 2005. Atmospheric CO2 uptake by a coastal upwelling system. *Global Biogeochem Cycles* 19(1).

Langdon CJ, Evans F, Jacobson D and Blouin, M. 2003. Yields of cultured Pacific oysters *Crassostrea gigas* Thunberg improved after one generation of selection. *Aquaculture*, 220:227-244.

American Society for Testing and Materials. 2004. Standard guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve molluscs. E724-98. 22 pp.

Data Processing Description

All the proportion normal data have been corrected to the control values for each experiment.

BCO-DMO edits:
- modified parameter names to conform with BCO-DMO naming conventions;
- replaced *C. gigas* and *M. galloprovincialis* with full names, *Crassostrea gigas* and *Mytilus galloprovincialis;*
- replaced missing data with "nd", meaning "no data".

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Data Files

File
oyster_mussel_carbonate.csv (Comma Separated Values (.csv), 5.05 KB) MD5:31351fea3ff28a64cd35cf6394e0758e Primary data file for dataset ID 638362

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Parameters

Parameter	Description	Units
species	Species of mussel or oyster.	dimensionless
experiment	Experiment identifier.	dimensionless
omega_trt	Treatment values for saturation state of aragonite are noted as "a" to "d" denoted the lowest to highest values.	dimensionless
CO2_trt	Treatment values for PCO2 are noted as Low (L), Mid-Low (ML), Mid_High (MH), and High (H).	dimensionless
alk	Alkalinity.	micromoles per kilogram (umol/kg)
DIC	Dissolved inorganic carbon (DIC).	micromoles per kilogram (umol/kg)
HCO3	Bicarbonate (HCO3).	micromoles per kilogram (umol/kg)
CO3	CO3	micromoles per kilogram (umol/kg)
PCO2	Partial pressure of carbon dioxide (PCO2).	micromoles per kilogram (umol/kg)
pH	pH.	pH scale
omega	Saturation state of aragonite.	dimensionless
fracN	Fraction of Normally Developed Larvae, proportion of normal larvae over all larvae.	dimensionless (proportion)
size	Size of shell.	micrometers (um)
fracN_stdev	Standard deviation of fracN.	dimensionless
size_stdev	Standard deviation of size.	micrometers (um)

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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	microscope
Generic Instrument Name	Microscope - Optical
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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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.

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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.

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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)

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Funding

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

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