

# Elemental compositions (Mg, Ca, Sr, N, C) of larval oyster shells from Whiskey Creek Shellfish Hatchery in Netarts Bay, OR, USA from 2009-2011

**Website:** <https://www.bco-dmo.org/dataset/639906>

**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)

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## Dataset Description

Elemental compositions (Mg, Ca, Sr, N, C) of larval oyster shells. Larvae were produced from adult oysters reared at Whiskey Creek Hatchery (WCH), Netarts Bay, Oregon, USA.

Related dataset: [Chemical composition of tank water](#)

## Methods & Sampling

### Sample Collection

Larvae were produced from adult oysters reared at Whiskey Creek Hatchery (WCH), Netarts Bay, Oregon, USA that were strip-spawned with the resultant sperm and eggs mixed in tanks filled with seawater collected from the bay and thermostatically controlled (to 22 degrees C). After hatching, larvae were grown for ~20 days, with a change in tank water every other day. Two cohorts were sampled, one in May and one in August, 2011. Details on the rearing methods can be found in Waldbusser et al., 2013. A fraction of the larvae and an aliquot of water coming from the bay to fill the rearing tanks were sampled during tank changes. The carbonate chemistry of the tank waters was also measured directly (DIC and PCO<sub>2</sub>, with calculated alkalinity and pH). Water was filtered at 0.2  $\mu$ m, collected in acid-washed bottles and acidified to pH ~2 with ultra pure HCl. The larvae were collected on a filter, rinsed with MilliQ water, then decanted and freeze-dried for later analyses.

## Water carbonate chemistry

Water filling the tanks is pumped directly from Netarts Bay, at an arbitrary time (i.e., not synchronized with tides). The salinity of these waters reflects that in the bay, but temperature is thermostatically-controlled (22 degrees C). The resulting carbonate chemistry is insignificantly modified from that in the bay due to the temperature change, aside from the effects on the carbonic acid equilibrium constants. For carbonate chemistry, all water samples were collected in 350 ml amber glass bottles, and sealed with polyurethane-lined metal crimp caps. PCO<sub>2</sub> 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, but modified for discrete samples. Standards for PCO<sub>2</sub> 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, the investigators used Millero's (2010) carbonic acid dissociation constants with temperature and salinity dependencies (which capture the Lueker et al. (2000) seawater constants with improved estimation at intermediate to low salinities), Dickson's (1990) constants for boric acid, and Millero's (1995) water dissociation constants. Combined uncertainty in the DIC and PCO<sub>2</sub> measurements is estimated at ~0.2% and ~2%, respectively, based on replicate analyses and comparison with Certified Reference Materials (Bockmon and Dickson, 2015). The Mucci (1983) aragonite solubility was used to calculate the aragonite saturation state ( $\Omega_a$ ) and pH was reported on the total hydrogen ion scale (pH<sub>T</sub>).

## Water metals chemistry (See [tank chemistry dataset](#))

Samples collected in pre-acid washed polypropylene bottles were analyzed on an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) at the OSU Keck Collaboratory. An IAPSO seawater and NIST 1643e seawater standard were run as external standards at concentrations equivalent to the water samples, and produced 2-sigma standard deviations ( $n = 5$  and  $n = 2$  respectively) of <3% for all elements analyzed (Ca, Mg, Sr). The metals of interest here (Ca, Mg, Sr) are conservative in the oceans, and their abundances measured in the tank water is generally equivalent to that predicted from salinity. The water of Day 13 in August is the only instance where the measured concentrations of Ca, Mg and Sr are significantly (>10%) different than what is expected from salinity. While the investigators' interpretations are based on the measured elemental concentrations, not salinity-based estimates, the generally good comparison indicates that there is no significant alteration of these metals in the transfer of water from the bay to the tanks.

## Shell Analyses

We transferred ~2 mg dry weight of freeze-dried larvae into an acid cleaned plastic 1.5 mL centrifuge vial. Our cleaning method was to treat each sample with a 5% (active chlorine) bleach solution. This reagent was added and reacted for 15 minutes, with constant agitation (shaking and sonication) to ensure reaction with all the larvae. The cleaning solution was then removed and the larval shells rinsed through shaking, centrifuging and pipette decanting with 5 repeats of MilliQ water. The cleaned, rinsed shells were then dissolved using ultra-pure 2 M nitric acid. These solutions were analyzed on a Thermo XSeries2 quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-MS), using an In-Re internal standard to correct for instrument drift. The external reproducibility of the analyses, based on replicate analyses of dilute NIST1643e standard was <4% for all analytes, and we report all ratios with a precision of 6%. The investigators further measured carbon and nitrogen content of the cleaned shells post-treatment. These samples were treated the same as described above, then dried in an oven at 60 degrees C before analyses using a Carlo Erba 1500 (Verardo et al., 1990). The precision of analyses for carbon and nitrogen in these carbonate samples is 3%. Finally, a fraction of these treated larval shells were dried for SEM imaging.

## References

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Millero F.J. 2010. Carbonate constants for estuarine waters. *Mar Freshwater Res.*, 61: 139-142. doi: [10.1071/MF09254](https://doi.org/10.1071/MF09254)

Mucci, A. 1983. The solubility of calcite and aragonite in seawater at various salinities, temperatures, and I atmosphere total pressure. *Amer. J. Sci.*, 238, pp. 780-799. doi: [10.2475/ajs.283.7.780](https://doi.org/10.2475/ajs.283.7.780)

Waldbusser, G.G., Brunner, E.L., Haley, B.A., Hales, B., Langdon, C.J. and Prahl, F.G. 2013. A developmental and energetic basis linking larval oyster shell formation to acidification sensitivity. *Geophys Res Lett.* 2013; 40: 2171-2176, doi: [10.1002/grl.50449](https://doi.org/10.1002/grl.50449)

## Data Processing Description

BCO-DMO Processing:

- modified parameter names to conform with BCO-DMO naming conventions;
- replaced 'n.m' (not measured) and blanks (missing data/not measured) with 'nd' (no data);
- 07 March 2018: removed embargo on dataset.

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## Data Files

File
<b>shell_compositions.csv</b> (Comma Separated Values (.csv), 633 bytes) MD5:e55cb51bc12f4e755eb546c4fcfd1055 Primary data file for dataset ID 639906

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## Parameters

Parameter	Description	Units
cohort	Cohort. Two cohorts were sampled, one in May and one in August 2011.	dimensionless
larval_age	Larval age in number of days.	number of days
length_mean	Mean length.	micrometers (um)
Mg_to_Ca	Ratio of molar concentration of Mg to Ca.	mmol/mol
Sr_to_Ca	Ratio of molar concentration of Sr to Ca.	mmol/mol
C_pcnt	Percent Carbon by weight.	% by weight
N_pcnt	Percent Nitrogen by weight.	% by weight

## Instruments

<b>Dataset-specific Instrument Name</b>	Tank
<b>Generic Instrument Name</b>	Aquarium
<b>Generic Instrument Description</b>	Aquarium - a vivarium consisting of at least one transparent side in which water-dwelling plants or animals are kept

<b>Dataset-specific Instrument Name</b>	amber glass bottles
<b>Generic Instrument Name</b>	Bottle
<b>Generic Instrument Description</b>	A container, typically made of glass or plastic and with a narrow neck, used for storing drinks or other liquids.

<b>Dataset-specific Instrument Name</b>	Carlo Erba 1500
<b>Generic Instrument Name</b>	Carlo-Erba NA-1500 Elemental Analyzer
<b>Generic Instrument Description</b>	A laboratory instrument that simultaneously determines total nitrogen and total carbon from a wide range of organic and inorganic sediment samples. The sample is completely and instantaneously oxidised by flash combustion, which converts all organic and inorganic substances into combustion products. The resulting combustion gases pass through a reduction furnace and are swept into the chromatographic column by the carrier gas which is helium. The gases are separated in the column and detected by the thermal conductivity detector which gives an output signal proportional to the concentration of the individual components of the mixture. The instrument was originally manufactured by Carlo-Erba, which has since been replaced by Thermo Scientific (part of Thermo Fisher Scientific). This model is no longer in production.

<b>Dataset-specific Instrument Name</b>	Thermo XSeries2 quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-MS)
<b>Generic Instrument Name</b>	Inductively Coupled Plasma Mass Spectrometer
<b>Generic Instrument Description</b>	An ICP Mass Spec is an instrument that passes nebulized samples into an inductively-coupled gas plasma (8-10000 K) where they are atomized and ionized. Ions of specific mass-to-charge ratios are quantified in a quadrupole mass spectrometer.

<b>Dataset-specific Instrument Name</b>	Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES)
<b>Generic Instrument Name</b>	Inductively Coupled Plasma Optical Emission Spectrometer
<b>Dataset-specific Description</b>	Samples collected in pre-acid washed polypropylene bottles were analyzed on an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) at the OSU Keck Collaboratory.
<b>Generic Instrument Description</b>	Also referred to as an Inductively coupled plasma atomic emission spectroscope (ICP-AES). These instruments pass nebulised samples into an inductively-coupled gas plasma (8-10000 K) where they are atomised and excited. The de-excitation optical emissions at characteristic wavelengths are spectroscopically analysed. It is often used in the detection of trace metals.

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## Deployments

### Waldbusser WCSH

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/541508">https://www.bco-dmo.org/deployment/541508</a>
<b>Platform</b>	Whiskey Creek Shellfish Hatchery
<b>Start Date</b>	2009-05-09
<b>End Date</b>	2011-08-01
<b>Description</b>	Whiskey Creek Shellfish Hatchery is a commercial shellfish hatchery located in Netarts Bay, a small bay on the northern Oregon coast.

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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 CO<sub>2</sub> 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 pCO<sub>2</sub>, 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 pCO<sub>2</sub> 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 californianus* 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 pCO<sub>2</sub> and dissolved inorganic carbon (DIC) instruments will be used in experiments to control and properly constrain the carbonate chemistry. A complement 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 CO<sub>2</sub>.

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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](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](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
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1041267</a>

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