

Biochemistry of 3 pacific oyster cohorts from Whiskey Creek Hatchery in Netarts Bay, OR, USA from 2009-2011

Website: <https://www.bco-dmo.org/dataset/639794>

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

Biochemistry summary of 3 pacific oyster cohorts from Whiskey Creek Hatchery. All data are presented in Table 4 of Brunner et al. (in review).

All details on sample collection, methods, and analytical techniques may be found in:

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

Brunner, E.L., F.G. Prah, B. Hales, G.G. Waldbusser (in review). Insights from Stable Isotopes into the Sensitivity of Larval Pacific Oysters to Ocean Acidification. *Marine Ecology Progress Series*.

The original file was split into 3 spreadsheets containing three different, but related datasets

[Isotope Data Summary](#)

Biochem Summary

[Carbonate Chemistry Summary](#)

Across all three datasets, there are 3 cohorts of pacific oyster larvae that have been sampled, one in May and two in August, one in untreated seawater, the other in buffered seawater. Time is all indexed to fertilization of the oysters, with samples collected on days noted.

Methods & Sampling

Methods for May Cohorts (from Waldbusser et al.)

The investigators measured the isotopic composition of carbon in larval shell, larval tissue, food, and holding tank water during growth and development of a commercial larval *Crassostrea gigas* cohort in May 2011. These measurements were coupled to bulk measures of larval biochemical composition to estimate energetic status and demands for the initial shell development.

Setting and Experimental Design

Crassostrea gigas larvae were raised according to commercial spawning protocols at Whiskey Creek Shellfish Hatchery in Netarts Bay, Oregon, as previously described in detail in Barton et al., 2012. Briefly, successfully fertilized Willapa Bay oyster larvae from several conditioned broodstock oysters were added to a 22 cubic meter tank an hour after fertilization at an estimated density of approximately 5×10^7 individuals per cubic meter. Water was changed in culture tanks every 2-3 days during cohort development. The pH of fertilization water was 8.15 (measured with a YSI 30 on the NBS scale), and developing embryos were reared under a P_{CO_2} of 396 uatm and aragonite saturation state of 2.38. Samples for P_{CO_2} and TCO_2 of culture tanks were collected at each tank filling and analyzed following Bandstra et al. (2006) with an uncertainty of less than 5% and 0.2% for P_{CO_2} and TCO_2 , respectively. The rest of the carbonate system was calculated using standard dissociation constants as previously described Barton et al., 2012. Conditions in culture tanks at filling had an average P_{CO_2} (± 1 S.D.) of 364 (± 40) uatm aragonite saturation state with of 2.58 (± 0.33) at the culture temperature of 25 degrees C, and a salinity of 26.5 (± 2.29). Larvae were fed a diet of *Isocrysis galbani* cultured in a continuous culture system during the first week following fertilization and this was switched to a mixed algae diet consisting primarily of *Chaetoceros gracilis*, *Thalassiosira spp.*, and *Isochrysis galbana* cultured in batches. Estimated cell densities were $5-7 \times 10^{10}$ cells per cubic meter.

During each tank change, all larvae were captured on an appropriately sized sieve and a primary sample of the entire cohort was randomly collected with a clean stainless steel spatula. The mass of each primary sample varied from ~0.5 to 1.0 g representing an estimated 2.8×10^6 to 5.6×10^6 two-day old larvae or 2.3×10^5 to 4.6×10^5 19-day old larvae. All 400 million larvae in the cohort were reared collectively in one tank, so replicate primary samples would have been pseudo-replicates of larvae from the same tank.

The larval samples were quickly rinsed with deionized water, immediately frozen, and freeze dried within 2 weeks with a Labconco FreeZone 6 Freeze Drier. Sub-samples from each primary sample were then used for subsequent analyses. Approximately 100's to 10's of larvae for stable isotope analyses of shell carbonates from Day 2 and 19 samples were needed, and lipid extractions required roughly 10^4 to 10^5 larvae for Day 2 and 19, respectively. Analyses of stable isotopes and elemental analyses of tissue required approximately 10^3 to 10^4 larvae. Variable volumes (200-800 ml) of algal cultures were vacuum filtered onto pre-combusted onto glass fiber filters (GF/F 47mm, Whatman) to achieve a similar analytical mass for all analyses.

$\delta^{13}C$ Measurements

All stable carbon isotope samples were analyzed in Oregon State University's CEOAS Stable Isotope Laboratory and details for standard analyses may be found at <http://stable-isotope.coas.oregonstate.edu/>. All runs were calibrated and checked with NIST certified (NBS 19, NBS 20 UQ6) and in-house calibrated standards, with precision and accuracy noted below.

Water samples were analyzed by continuous mass flow spectrometry on a GasBench-Delta V system. The run had a precision of 0.02‰ and accuracy better than 0.06‰. Larval shell carbonate $\delta^{13}C$ was measured by reacting the freeze-dried larvae with a viscous ~105% H_3PO_4 solution in a Finnigan MAT Kiel III at 70 degrees C for 5 minutes after which the isotopic composition of the evolved and purified CO_2 was measured on a dual inlet mass spectrometer (MAT 252 IRMS). Each run had a precision and accuracy of 0.02‰ and 0.01 ‰, respectively. Repeatability of larval shell samples was within 0.02‰. To obtain measures of tissue $\delta^{13}C$ composition of tissues, freeze-dried larvae acid fumed with concentrated HCl to eliminate the shell carbonate signal. The remaining sample material was then analyzed by flash combustion (>1000 degree C) using a Carlo Erba NA 1500 elemental analyzer interfaced by continuous-flow mass spectrometer (Delta-Plus XL). Repeatability of organic larval samples was 0.21‰. Algae samples, collected as noted above, were analyzed in the same way as larval tissue however without the acid fuming. Measurements of organic $\delta^{13}C$ composition were made with a precision and accuracy of 0.17‰ and 0.12‰, respectively.

Larval Biochemical and Size Analyses

Fractions of organic tissue and shell (% of dry weight) were determined by first measuring %C on bulk freeze-dried larval samples and then decalcified samples (via HCl fuming) on a Carlo Erba NA1500 elemental analyzer. The difference between whole and decalcified larvae and the molar proportion of C in calcium carbonate were

used to estimate the % calcium carbonate by weight, with the remainder being organic matter. These values for % organic matter were correlated to ($r^2 = 0.89$) loss on ignition (LOI) measurements, with LOI consistently over estimating % organic matter. Total lipid % was measured by extracting freeze-dried larvae with a methylene chloride: methanol solution (3:1) using a Dionex ASE 200 Accelerated Solvent Extractor and weighing an aliquot of the isolated total extractable lipid (TEL) fraction dissolved in CS_2 gravimetrically. Larval size was evaluated on 100-150 larvae per sample, taken as a separate sub-sample during tank water changes and evaluated on digital micrographs using standard image analysis techniques (ImageJ 1.44p). A standard mass at length model for *C. gigas* larvae (Bocheneck *et al.*, 2001) was used to calculate a total individual mass. Measured proportional composition data were then applied to the mass at length estimate to compute per larva weights of shell, bulk organic, and lipid content.

Methods for August Cohorts (from Brunner *et al.*)

The investigators sampled multiple commercial cohorts of Pacific oyster larvae, *Crassostrea gigas*, at the Whiskey Creek Shellfish Hatchery (WCH), Netarts, Oregon, U.S.A. where carbonate chemistry within the hatchery is set by the ambient Netarts Bay conditions, but food availability (*ad libitum*) and temperature (25 degrees C) are controlled to optimal levels. The large (~400 million larvae) cohorts provided sufficient larvae to measure bulk biochemistry parameters such as organic content and composition while the sequential tank incubations allowed us to fully constrain the possible isotopic sources for incorporation into shell and tissue at discrete intervals during larval life (e.g., 0-2 days, 2-5 days).

Setting and Experimental Design

The investigators followed three cohorts of approximately 400 million *Crassostrea gigas* larvae from fertilization through metamorphosis: one cohort in May 2011, and two in August 2011. WCH's spawning protocols are described in detail by Barton *et al.* (2012), and by Waldbusser *et al.* (2013). Briefly, larvae are maintained in static 22 cubic meter tanks, which have their water changed every 2-3 days, and are fed daily. The water for each tank change is pumped from Netarts Bay — a marine-dominated estuary on the northern Oregon coast (45.403N, 123.944W) which is flushed nearly every tidal cycle with water from the adjacent North Pacific Ocean (Whiting & McIntire 1985). With roughly two-thirds of the 9.41 square-kilometer aerial extent covered in *Zostera spp.* beds, and the remaining tidal flats supporting microphytobenthos production, there is a diurnal PCO_2 cycle that reflects the daily cycle of photosynthesis and respiration in Spring and Summer such that the highest PCO_2 values are seen in the morning after a night of respiration and the lowest PCO_2 values are seen in the afternoon (Waldbusser & Salisbury 2014). Thus, the saturation state at the hatchery intake pipe is variable on hourly, weekly and seasonal timescales (Barton *et al.* 2012, Waldbusser & Salisbury 2014). To exaggerate the naturally-occurring PCO_2 conditions of the upwelling season in August, water was pumped in the morning. May 2011 was a period of high freshwater input into Netarts Bay and thus the water started with a lower salinity (~26) and thus alkalinity than usual. For the May cohort, water for each tank-fill was pumped in the afternoon, when PCO_2 was relatively low. All water is heated to 25 degree C before being pumped into the tanks.

Spawn and Larval Culture

On 18 May 2011 and again on 14 August 2011 at least 3 Willapa Bay females were strip-spawned and fertilized with sperm from at least one Willapa Bay male. The fertilized eggs in August were split into two different tanks ~ 1 hr after fertilization (a "buffered" and control, each starting with approximately 400 million larvae), and followed separately for the remainder of their larval period. The WCH buffer system treated incoming water with industrial grade soda ash (Na_2CO_3) to an aragonite saturation state (Ω_{ar}) equal to 4 during this experiment.

All larvae were fed a rotating mixture of algal cultures grown at WCH including *Isochrysis sp.*, *Chaetoceros gracilis*, and *Chaetoceros calcitrans*. In the first 5-10 days the larvae were fed *Isochrysis sp* from a continuous culture bag system and these algae have a particularly low $\delta^{13}C$ signal [around -45‰] which is due to the bubbling of culture water with compressed industrial grade CO_2 . At each feeding, between 200 and 800 mL of algal feed cultures were filtered onto pre-combusted 47mm GF/F glass fiber filters (Whatman) and the algae retained on the filter dried at <45 degrees C for storage until analysis. The sample volume was determined to account for different densities of algal culture and achieve optimal amounts of organic material on each filter for analysis.

At each tank water change, the previous tank's water was drained onto an appropriately sized sieve to capture all the larvae, and the larvae were then transferred into a newly-filled tank. A 0.5 - 2 g dry weight sub-sample (representing an estimated $3-10 \times 10^6$ two-day old larvae or $2-9 \times 10^5$ 19-day old larvae) of the cohort was collected from the sieve, briefly rinsed with DI water and immediately frozen. The samples were then freeze-dried within three weeks of collection (Labconco FreeZone 6 Freeze Drier). As all the larvae from each cohort

were living in the same tank, taking multiple primary samples would have been pseudo-replication, and the investigators therefore assume that the sub-samples accurately represent the entire larval cohort. In May, after 5 days, the fastest growing larvae were selected by passing the cohort through an 80 μm sieve, which caught about half of the larvae. This procedure for poorly performing cohorts commonly employed at WCH. The August cohorts were not sorted via this size-fraction screening as their general growth and fitness was better.

Isotopic and Compositional Measurements of Larvae and Algae

All stable carbon isotope samples were analyzed in Oregon State University's College of Earth, Ocean, and Atmospheric Sciences Stable Isotope Laboratory. Detailed descriptions of these standard protocols can be found in Waldbusser et al. (2013) and at <http://stable-isotope.coas.oregonstate.edu/>. All runs were calibrated and checked with NIST certified and in-house calibrated standards. Precision and accuracy are noted for each analysis.

To measure larval tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, 6-10 mg freeze-dried larvae in Ag boats were acidified in a concentrated HCl atmosphere following the method of Hedges & Stern (1984). The samples were dried overnight, encapsulated in Sn boats, and loaded into a Costech Zero Blank Autosampler. Samples were flash combusted at >1020 degrees C using a Carlo Erba NA1500 Elemental Analyzer (Verardo et al. 1990) and the resulting gas was analyzed by continuous-flow mass spectrometry using a DeltaPlus XL isotope ratio mass spectrometer. Hole punches of filter (diameter $\sim 7\text{mm}$) with dried algae were analyzed via the same method as the larval tissue, without the acidification step. Repeatability of samples was 0.21‰ for $\delta^{13}\text{C}$, and 0.27‰ for $\delta^{15}\text{N}$.

In order to measure larval shell carbonate $\delta^{13}\text{C}$, the investigators reacted 19-50 freeze-dried larvae with concentrated phosphoric acid at 70 degrees C for 5 minutes in a Finnigan Keil III device. The resulting CO_2 was isolated from other reaction products and measured on a MAT 252 mass spectrometer by dual inlet mass spectrometry. Accuracy was better than $\pm 0.05\text{‰}$, and repeatability was $\pm 0.02\text{‰}$.

Larval samples were also analyzed for organic and inorganic elemental composition before and after acidification as described above on a Carlo Erba NA-1500 elemental analyzer calibrated with at least 6 acetanilide standards following the methods of Verardo et al. (1990). Ash-free dry weight (AFDW) was determined by weight comparison before and after combustion at 490 degrees C for 4 hours.

Extractable Lipids, Length and SEM Imaging

At each tank change, an aliquot of larvae were fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in a 0.1 M cacodylate buffer. Samples were later transferred to 50% ethanol and, imaged using an Olympus SZH10 dissecting microscope. 100 larval lengths per sampling point were measured (ImageJ 1.44p). Lengths were converted into weights using the dry-tissue weight to length relationship for larval *C. gigas* from Bochenek et al. (2001), accounting for our measured proportion shell (1-AFDW) at each time point, instead of the 75% shell assumption used by the model.

To measure extractable lipids, ~ 75 mg samples of freeze-dried larvae were extracted on a Dionex ASE 200 Accelerated Solvent Extractor for four cycles of 5 minutes at 1500 psi with 3:1 methylene chloride:methanol. The extracts were combined with 25 mL hexane and washed against 10 mL 50% saturated NaCl to remove hydrophilic impurities (proteins, carbohydrates). The aqueous phase was drained away and the lipid-containing fraction was dried under an N_2 gas stream. The lipid concentrate was then re-suspended in 500 μL CS_2 and an aliquot (100 μL) moved to a tared nickel weighing boat, dried and re-weighed to get a gravimetric weight of lipid. This weight was compared to the original sample weight to find the fraction total lipid. As with the other bulk measurements, these values were normalized to the larval weights to estimate lipid (ng) per larvae. Additionally, it should be noted that the lipid measurements by Waldbusser et al. (2013) may be an over-estimate, due to the gravimetric extraction method used. The washed values presented here provide a better estimate of the true lipid content. Due to a sampling error, the investigators do not have a reliable washed lipid value for the 48 hour time point for the May cohort. Given the similarity between the washed and unwashed values in early life, the unwashed gravimetric lipid weight (as reported in Waldbusser et al. 2013) are used instead.

Carbonate chemistry

ater samples were collected at each tank filling prior to the addition of larvae or algae. For dissolved inorganic carbon (DIC) ^{13}C analysis, aliquots of HgCl_2 -fixed sample were pipetted into Labco glass vials sealed with rubber septa caps. The vials were cooled to 13 degrees C, allowed to equilibrate for 15 minutes, and purged with He for 5 minutes each. 85% phosphoric acid was then added to each vial via syringe. The samples were left to equilibrate for 10 hours and analyzed via continuous-flow mass spectrometry using a GasBench-DeltaV

system. Each run was standardized using a combination of sodium bicarbonate (3 mM in solution) and calcium carbonate standards. These methods have a precision of 0.15 ‰ and repeatability better than 0.2 ‰.

Samples for PCO₂ and dissolved inorganic carbon (DIC) analysis were collected at each water change in 330 mL amber glass bottles, poisoned with HgCl₂ and analyzed following Bandstra et al. (2006) The uncertainty of these measurements is less than 2% and 0.2% for PCO₂ and DIC, respectively. The remaining carbonate system parameters were calculated using the carbonic acid dissociation constants of Millero (2010), the boric acid constants as defined by Dickson (1990), and the aragonite solubility as defined by Mucci (1983).

Data Processing Description

The derived variables in the Biochemistry dataset are Est. Weight (weight_est), Est. Lipid (lipid_est), Tissue atomic C:N (tissue_C_to_N), and calcification rate (calcif_rate). These are computed as follows:

Estimated Weight - computed from Bochenek et al. (2001) weight at size model, initially assuming fixed proportion of organic and inorganic components to compute total weight.

Total weight = $(6.745 \times (10^{-4}) \times (\text{shell length}^{2.557})) \times 4$

Est Lipid - fraction lipid multiplied by estimated total weight.

Tissue atomic C:N is the molar ratio of tissue carbon and nitrogen.

Calcification rate is computed by the change in shell mass divided by number days between measurements.

BCO-DMO Processing:

- modified parameter names to conform with BCO-DMO naming conventions;
- replaced blanks (missing data) with "nd", meaning "no data";
- replaced "Aug" with "August" in cohort name column;
- 04-April-2018: removed embargo on dataset.

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Data Files

File
biochem.csv (Comma Separated Values (.csv), 2.14 KB) MD5:51eb0480afc91aedbb82aa6b55dbb360 Primary data file for dataset ID 639794

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Parameters

Parameter	Description	Units
cohort	There are 3 cohorts of pacific oyster larvae that have been sampled, one in May and two in August, one in untreated seawater, the other in buffered seawater.	dimensionless
days_since_fert	Days since fertilization. Time is indexed to fertilization of the oysters, with samples collected on days noted.	number of days
lipid_frac	Fraction lipid (wt/wt)	wt/wt
organic_frac	Fraction organic (AFDW)	ash-free dry weight (AFDW)
length_mean	Mean length	micrometers (um)
length_stdev	Standard deviation of length.	micrometers (um)
weight_est	Estimated weight	nanograms per larva (ng larva ⁻¹)
lipid_est	Estimated lipid	nanograms per larva (ng larva ⁻¹)
total_frac_N	Total fraction Nitrogen (wt/wt)	wt/wt
total_frac_C	Total fraction Carbon (wt/wt)	wt/wt
organic_frac_N	Organic fraction Nitrogen (wt/wt)	wt/wt
organic_frac_C	Organic fraction Carbon (wt/wt)	wt/wt
tissue_C_to_N	Tissue atomic C:N ratio	ratio
calcif_rate	Calcification rate	per day (day ⁻¹)

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Instruments

Dataset-specific Instrument Name	Carlo Erba NA 1500
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	The remaining sample material was then analyzed by flash combustion (>1000 degrees C) using a Carlo Erba NA 1500 elemental analyzer interfaced by continuous-flow mass spectrometer (Delta-Plus XL).
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	isotope ratio mass spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	May cohorts: Larval shell carbonate $\delta^{13}\text{C}$ was measured by reacting the freeze-dried larvae with a viscous ~105% H_3PO_4 solution in a Finnigan MAT Kiel III at 70 degrees C for 5 minutes after which the isotopic composition of the evolved and purified CO_2 was measured on a dual inlet mass spectrometer (MAT 252 IRMS). August cohorts: Samples were flash combusted at >1020 degrees C using a Carlo Erba NA1500 Elemental Analyzer (Verardo et al. 1990) and the resulting gas was analyzed by continuous-flow mass spectrometry using a DeltaPlus XL isotope ratio mass spectrometer.
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	continuous-flow mass spectrometer
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Water samples were analyzed by continuous mass flow spectrometry on a GasBench-Delta V system. The remaining sample material was then analyzed by flash combustion (>1000 degrees C) using a Carlo Erba NA 1500 elemental analyzer interfaced by continuous-flow mass spectrometer (Delta-Plus XL).
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

Dataset-specific Instrument Name	YSI 30
Generic Instrument Name	pH Sensor
Dataset-specific Description	The pH of fertilization water was measured with a YSI 30.
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^+).

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Deployments

Waldbusser_WCSH

Website	https://www.bco-dmo.org/deployment/541508
Platform	Whiskey Creek Shellfish Hatchery
Start Date	2009-05-09
End Date	2011-08-01
Description	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 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 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 pCO₂ 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 CO₂.

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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