Experimental Ocean Carbonate System Controls on Mytilus trossulus Shell Growth from 2023 to 2024

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Data Type: experimental
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Project
» Invertebrate calcification and behavior in seawater of decoupled carbonate chemistry (OA decoupling)

Contributors	Affiliation	Role
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Abstract
This dataset contains experimental carbonate chemistry manipulations and calcification responses in adult Mytilus trossulus, conducted between September 2023 and February 2024. Mussels were collected from Penn Cove Shellfish Farm in Penn Cove, WA, a coastal environment influenced by freshwater input. The experiment used multiple treatment types designed to decouple carbonate system parameters commonly altered by freshwater runoff, which influences seawater carbonate chemistry through changes in total alkalinity (TA) and dissolved inorganic carbon (DIC), with subsequent shifts in pH, $[HCO_3^-]$, $[CO_3^{2-}]$, and saturation state (Ω). The dataset includes seawater carbonate chemistry conditions (e.g., pH, total alkalinity (TA), DIC, $[HCO_3^-]$, pCO_2, Ω, and $[Ca^{2+}]$) and biological response measurements of calcification and dissolution rates. Experimental treatments included: (1) multiple treatments spanning low-to-high pH and $[HCO_3^-]$; (2) a range of saturation states under high and low $[HCO_3^-]$ conditions using both live mussels and de-fleshed mussel shells; and (3) calcium manipulations to distinguish the relative influence of pH versus Ω on calcification by altering $[Ca^{2+}]$ independently of pH. This dataset describes treatment conditions, shell and tissue mass, carbonate system calculations, and calcification and dissolution rates calculated using the ammonia-corrected alkalinity anomaly technique (Gazeau et al. 2013). Future researchers can use this data to examine the effect of freshwater-influenced seawater conditions on calcification responses in coastal calcifying organisms. Datasets are provided in .csv format for maximum accessibility and interoperability.

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Coverage

Location: Penn Cove, Skagit Bay, Washington, USA
Temporal Extent: 2023-09 - 2024-02

Methods & Sampling

Adult Mytilus trossulus (44–75 mm shell length) were collected in September 2023 (n = 131) and December 2023 (n = 73) from Penn Cove Shellfish Farm in Penn Cove, WA, USA. Once they arrived at UC Davis Bodega Marine Lab, they were acclimated for 10 days in flow-through seawater tanks maintained at approximately 13°C prior to experimentation. The following methods are based on and derived from the protocol described in Ninokawa et al. (2024) and Carlson et al. (2025). Experimental incubations were done in a temperature-controlled room at 13°C using airtight 1 L glass vessels containing approximately 0.86 kg seawater and either a live mussel, a de-fleshed and sterilized shell, or only seawater (blank controls to account for any background changes in seawater chemistry).

The experiment consisted of three complementary carbonate chemistry manipulation approaches intended to separate the effects of pH, bicarbonate concentration ([HCO_3^-]), calcium carbonate saturation state (Ω), and calcium concentration ([Ca^{2+}]) on calcification and dissolution rates. To make each treatment, 20 L sumps of filtered seawater were first acidified with Hydrochloric acid (HCl), reducing total alkalinity and shifting dissolved inorganic carbon (DIC) equilibrium toward dissolved CO_2. The seawater was then aerated for ~48hrs with Tetra Whisper AP300 Air Pumps to remove excess CO_2 through off-gassing. Following aeration, a gradient-based treatment design targeted a continuum of carbonate chemistry conditions, achieved through varying additions of DIC stock solution, HCl, Sodium hydroxide (NaOH), Calcium chloride ($CaCl_2$), or Sodium chloride (NaCl). Carbonate chemistry targets were planned using the R package *seacarb*.

For the first trial, we assessed how pH and DIC influence calcification processes using a batch-treatment design of nine manipulated carbonate chemistry treatments spanning low-to-high pH (7.1–8.2) and [HCO_3^-] (878.5–4256.8 µmol/kg) conditions, along with one ambient seawater control treatment. For this portion of the experiment, each treatment consisted of a 20L seawater chemistry batch that was distributed among nine replicate live-mussel incubation vessels.

A second trial quantified abiotic shell dissolution across a broad calcium carbonate saturation state (aragonite) gradient (0.05–8.24) using de-fleshed mussel shells that had been sterilized by drying at 60°C for approximately 24 h prior to incubation. These shell-only incubations (n = 81) allowed the disaggregation of net calcification from dissolution in order to estimate gross calcification rates (gross calcification = net calcification + dissolution). In contrast to the live-mussel incubations, shell-only incubations isolated abiotic calcium carbonate dissolution from physiological processes such as respiration and ammonium excretion. To increase the magnitude of alkalinity changes associated with shell dissolution, dissolution incubations done in December 2023 and February 2024 were extended to 42–44 h, compared to the 2–4 incubations done in September 2023.

In the third trial, we independently manipulated calcium concentration to decouple Ω from pH. This experiment used a 2 × 2 factorial design with two target pH conditions (approximately 7.8 and 8.1) and two calcium treatments: ambient Ω (“low Ca^{2+}”, adjusted with NaCl) and elevated Ω (“high Ca^{2+}”, adjusted with $CaCl_2$). Addition of $CaCl_2$ increased Ω without substantially altering pH to differentiate whether calcification responses were more strongly associated with Ω or pH under similar bicarbonate conditions. Each calcium treatment combination contained six replicate live-mussel incubation vessels.

For all experiments, seawater chemistry measurements were collected before and after incubations. Measurements included total alkalinity, pH, dissolved oxygen, salinity, temperature, and ammonia concentration; calcium concentration was additionally measured for calcium-manipulation treatments. Initial and final alkalinity were analyzed using a Metrohm robotitrator. A salicylate spectrophotometric assay measured ammonia concentration to correct alkalinity anomaly calculations for any alkalinity changes caused by ammonium production rather than calcification.

Data Processing Description

Net calcification rates were determined using the ammonia-corrected alkalinity anomaly technique and normalized to incubation duration, seawater mass (kg), and tissue dry mass (Gazeau et al 2015). Abiotic shell dissolution was quantified from parallel incubations of cleaned, sterilized shells and added to net calcification rates to determine rate of gross calcification. Data processing was done with R statistical software, version 4.3.3. Carbonate system parameters were calculated using the package *seacarb* and equilibrium constants from Lueker et al 2000.

BCO-DMO Curation Notes

* Loaded Mytilus_trossulus_carbonate_decoupling_incubation_data.csv as table res1, using "", "NA", and "nd" as missing values.
* Removed a blank-named column.
* Renamed columns for consistency, including mussel.number to mussel_number, h2o.kg to h2o_kg, date.cat to date_cat, and treatment to sample_type.
* Added scientific_name, assigning Mytilus trossulus where applicable.
* Added the WoRMS identifiers for Mytilus trossulus: AphiaID 140482 and LSID urn:lsid:marinespecies.org:taxname:140482. These values were hard-coded because live API lookups were unavailable.
* Reordered the columns into the final specified order.
* Set column data types, including integers for mussel_number and aphia_id, numbers for measurement fields, and strings for descriptive and categorical fields.
* Added metadata—including descriptions, standard names, and units—for all columns. Units included µmol/kg, unitless, degrees C, hours, and g, as applicable.
* Saved the final data file as 1007055_1_data.csv.

Problem Description

No problems or issues have been reported by the dataset authors.

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Related Publications

Carlson, R. R., Lewis, M. A., Ninokawa, A. T., Saley, A. M., Hill, T. M., & Gaylord, B. (2025). Shell dissolution rates differ fourfold between mussel species. Royal Society Open Science, 12(7). https://doi.org/10.1098/rsos.250664
Results

Gazeau, F., Parker, L. M., Comeau, S., Gattuso, J.-P., O'Connor, W. A., Martin, S., Pörtner, H.-O., & Ross, P. M. (2013). Impacts of ocean acidification on marine shelled molluscs. Marine Biology, 160(8), 2207–2245. https://doi.org/10.1007/s00227-013-2219-3
Methods

Gazeau, F., Urbini, L., Cox, T., Alliouane, S., & Gattuso, J. (2015). Comparison of the alkalinity and calcium anomaly techniques to estimate rates of net calcification. Marine Ecology Progress Series, 527, 1–12. https://doi.org/10.3354/meps11287
Methods

Lueker, T. J., Dickson, A. G., & Keeling, C. D. (2000). Ocean pCO2 calculated from dissolved inorganic carbon, alkalinity, and equations for K1 and K2: validation based on laboratory measurements of CO2 in gas and seawater at equilibrium. Marine Chemistry, 70(1-3), 105–119. https://doi.org/10.1016/s0304-4203(00)00022-0 https://doi.org/https://doi.org/10.1016/S0304-4203(00)00022-0
Methods

Ninokawa, A. T., Saley, A. M., Shalchi, R., & Gaylord, B. (2024). Multiple carbonate system parameters independently govern shell formation in a marine mussel. Communications Earth &Amp; Environment, 5(1). https://doi.org/10.1038/s43247-024-01440-5
Methods

Orr, J., Gattuso, J.-P., Epitalon, J.-M., Lavigne, H., Gentili, B., Hagens, M., Hofmann, A., Mueller, J.-D., Proye, A., Rae, J., & Soetaert, K. (2026). seacarb: Seawater carbonate chemistry (Version 3.4.1) [R package]. Comprehensive R Archive Network. https://doi.org/10.32614/CRAN.package.seacarb https://CRAN.R-project.org/package=seacarb
Software

Saley, A., & Gaylord, B. (2024). *Lab incubations of mussels (Mytilus californianus) examining the influence of periostracum cover and pH on external shell dissolution at Marshall Gulch Beach, CA from August 2021 to March 2022* (Version 1) [Dataset]. Biological and Chemical Oceanography Data Management Office (BCO-

DMO). https://doi.org/10.26008/1912/BCO-DMO.935476.1 https://doi.org/10.26008/1912/bco-dmo.935476.1
Methods

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Parameters

Parameter	Description	Units
mussel_number	Unique ID assigned to each mussel or shell.	unitless
species	Mussel species used in experiment.	unitless
scientific_name	Mussel species used in experiment in scientific name format validated by the WORMS database.	unitless
aphia_id	Aphia ID for the Mussel species used in the experiment, matched from the WORMS database.	unitless
lsid	LSID for the Mussel species used in the experiment, matched from the WORMS database.	unitless
date_cat	Month and year at which experiment was conducted.	unitless
sample_type	Treatments were done on either live" mussels or dissected "shells". "	unitless
duration	Incubation period.	hours
sal	Treatment water salinity.	PSU
temp	Treatment water temperature (measured by Horiba Laqua PC1100).	degrees Celsius
gross_calc	Gross calcification rate calculated for live" mussels as: ((-0.5 * (del_ta - del_nh3) * h2o.kg) - calc_diss) /duration/ tissue_mass."	umol/kg/hr
abiotic_diss	Abiotic dissolution rate for shells" calculated as (del_ta* (h20.kg)/2/duration/shell_mass)."	umol/kg/hr
tissue_mass	Mass of dried shell tissue.	g
shell_mass	Mass of dried shell.	g

TA	Average total alkalinity during incubation (measured by Metrohm 855 Robotic Titrosampler).	umol/kg
pH_t	pH on total scale during incubation (measured by Horiba Laqua PC1100).	unitless
hco3	Bicarbonate concentration during incubation (derived from TA and pH using seacarb: Seawater Carbonate Chemistry R package).	umol/kg
co3	Carbonate concentration during incubation (derived from TA and pH using seacarb: Seawater Carbonate Chemistry R package).	umol/kg
OmegaAragonite	Aragonite saturation state during incubation (derived from TA and pH using seacarb: Seawater Carbonate Chemistry R package).	unitless
OmegaCalcite	Calcite saturation state during incubation (derived from TA and pH using seacarb: Seawater Carbonate Chemistry R package).	unitless
pH_free	Free scale pH during incubation (derived from TA and pH using the seacarb: Seawater Carbonate Chemistry R package).	unitless
H_free	Hydrogen ion concentration (derived from TA and pH using seacarb: Seawater Carbonate Chemistry R package).	unitless
SIR	Substrate Inhibitor Ratio during incubation (derived from TA and pH using seacarb: Seawater Carbonate Chemistry R package).	unitless
co2	Carbon dioxide concentration during incubation (derived from TA and pH using seacarb: Seawater Carbonate Chemistry R package).	umol/kg
pCO2	Partial pressure of carbon dioxide during incubation (derived from TA and pH using seacarb: Seawater Carbonate Chemistry R package).	uatm
DIC	Total Dissolved Inorganic Carbons during incubation ([CO2] + [HCO3-] + [CO32-]), (derived from TA and pH using seacarb: Seawater Carbonate Chemistry R package).	umol/kg
h2o_kg	Mass of seawater in individual incubation vessel (derived from TA and pH using seacarb: Seawater Carbonate Chemistry R package).	kg
del_ta	Change in total alkalinity before and after treatment (derived from TA and pH using seacarb: Seawater Carbonate Chemistry R package).	umol/kg
del_nh3	Change in NH3 concentration before and after treatment (derived from TA and pH using seacarb: Seawater Carbonate Chemistry R package).	umol/kg

calc_diss	Correction for alkalinity change due to abiotic dissolution, calculated by dividing $CaCO_3$ dissolution by incubation time and dry shell mass. Only applies to live" mussels. "	umol/kg

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Instruments

Dataset-specific Instrument Name	PreSens Microx 4 Micro-optode
Generic Instrument Name	Optode
Dataset-specific Description	Oxygen and temperature were measured using a PreSens Microx 4 micro-optode; salinity with a Horiba Laqua PC 1100 conductivity probe; and pH with a Horiba Laqua PC 1100 instrument.
Generic Instrument Description	An optode or optrode is an optical sensor device that optically measures a specific substance usually with the aid of a chemical transducer.

Dataset-specific Instrument Name	Shimadzu Spectrophotometer
Generic Instrument Name	Spectrometer
Dataset-specific Description	To conduct ammonia and pH analysis were done with a Shimadzu Spectrophotometer.
Generic Instrument Description	A spectrometer is an optical instrument used to measure properties of light over a specific portion of the electromagnetic spectrum.

Dataset-specific Instrument Name	Metrohm 855 Robotic Titrosampler
Generic Instrument Name	Titrator
Dataset-specific Description	A Metrohm 855 Robotic Titrosampler, an 800 Dosino, and an 856 Conductivity Module were used in tandem to measure Total Alkalinity before and after treatment.
Generic Instrument Description	Titrators are instruments that incrementally add quantified aliquots of a reagent to a sample until the end-point of a chemical reaction is reached.

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Project Information

Invertebrate calcification and behavior in seawater of decoupled carbonate chemistry (OA decoupling)

Coverage: California coast, USA

NSF Award Abstract:
This research is exploring the capacity of coastal organisms to cope with alterations in seawater chemistry driven by both freshwater inputs and absorption of carbon dioxide into the world's oceans (ocean acidification). The project focuses on calcification responses and behavioral impairments of shoreline animals under altered seawater chemistry, and forefronts a common mussel species (the California mussel), and a common snail (the black turban snail), each abundant on rocky shores along the west coast of North America. The target species operate as exemplar organisms for characterizing the responses of marine invertebrates more generally. Methods involve experimental decoupling of multiple components of the carbonate system of seawater to isolate drivers that are difficult to separate otherwise. Broader impacts include transfer of scientific information to policy-makers, including legislators, as well as training and skill-set development of future generations of scientists and citizens. One Ph.D. student is supported, as are UC Davis undergraduates conducting mentored research. The project also provides research internships for undergraduates from a local community college (Santa Rosa Junior College), many of whom are from underrepresented groups. The latter project component substantially bolsters an ongoing program at Bodega Marine Laboratory that includes efforts in diversity, equity, and inclusion. Data and interpretations from the project are feeding into an existing educational program that links to local K-12 schools and reaches ~10,000 members of the public each year.

Overall, the research of the project is dissecting drivers of calcification and behavioral disruption in key shoreline invertebrates, across present-day and future carbonate system conditions appropriate to coastal marine environments. Efforts are exploring the extent to which calcification depends on one versus multiple parameters of the seawater carbonate system. In particular, existing conceptual models emphasize the importance of calcium carbonate saturation state (Ω) and/or the ratio of bicarbonate to hydrogen ion concentrations ($[HCO_3^-]/[H^+]$), and the project is examining these mechanisms as well as the possibility that more than one driver acts simultaneously. It is doing so both in bivalves and in gastropods to test for generality across mollusks. The project is additionally examining whether pH is the only carbonate system factor contributing to known patterns of behavioral impairment in marine invertebrates. Leading explanations for debilitating behaviors induced by ocean acidification involve altered ion channel function, but discussion in the literature continues, and studies that explicitly decouple the carbonate system are necessary.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2129942

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