

Incubation data for *Mytilus californianus* calcification from January to April 2022 (OA decoupling project)

Website: <https://www.bco-dmo.org/dataset/925598>

Data Type: Other Field Results, experimental

Version: 1

Version Date: 2024-05-06

Project

» [Invertebrate calcification and behavior in seawater of decoupled carbonate chemistry](#) (OA decoupling)

Contributors	Affiliation	Role
Gaylord, Brian	University of California-Davis (UC Davis)	Principal Investigator
Ninokawa, Aaron T.	University of California-Davis (UC Davis-BML)	Student, Contact
Saley, Alisha	University of California-Davis (UC Davis-BML)	Student
Shalchi, Roya	University of California-Davis (UC Davis-BML)	Student
Newman, Sawyer	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract

Calcification is vital to marine organisms that produce calcium carbonate shells and skeletons. However, how calcification is impacted by ongoing environmental changes, including ocean acidification, remains incompletely understood due to complex relationships among the carbonate system variables hypothesized to drive calcification. Here, we experimentally decouple these drivers in an exploration of shell formation in adult marine mussels, *Mytilus californianus*. In contrast to models that focus on single parameters like calcium carbonate saturation state, our results implicate two independent factors, bicarbonate concentration and seawater pH, in governing calcification. While qualitatively similar to ideas embodied in the related substrate-inhibitor ratio (bicarbonate divided by hydrogen ion concentration), our data highlight that merging bicarbonate ion and hydrogen ion concentrations into a simple quotient obscures important features of calcification. Considering a dual-parameter framework improves mechanistic understanding of how calcifiers interact with complex and changing chemical conditions.

Table of Contents

- [Coverage](#)
- [Dataset Description](#)
 - [Methods & Sampling](#)
 - [Data Processing Description](#)
 - [BCO-DMO Processing Description](#)
- [Data Files](#)
- [Related Publications](#)
- [Related Datasets](#)
- [Parameters](#)
- [Instruments](#)
- [Project Information](#)
- [Funding](#)

Coverage

Location: Laboratory experiments were conducted at the Bodega Marine Laboratory in Bodega Bay, CA, USA and specimens were collected from Carmet Beach

Spatial Extent: **Lat:**38.371361 **Lon:**-123.076306

Temporal Extent: 2022-01-13 - 2022-04-23

Dataset Description

Each row of the primary file of this dataset corresponds to incubation data collected while exposing an individual mussel to modified seawater.

Methods & Sampling

Sampling collection details

We gathered naturally settled, adult California mussels (*M. californianus* between 30 and 80 mm in maximum shell length) by hand from the mid-intertidal zone of Carmet Beach, along the northern California coast. We cleaned mussels of all epibionts and external byssal threads, then transported them in buckets (< 0.5 hr transit time) to Bodega Marine Laboratory, where we acclimated individuals for seven days in flow-through seawater tables prior to subsequent experiments.

Experiment details

Incubations conducted according to published methods (Gazeau et al 2015).

Seawater chemistry was controlled to target unique combinations of alkalinity (between 337 and 9584 $\mu\text{mol kg}^{-1}$) and dissolved inorganic carbon (DIC, between 299 and 9322 $\mu\text{mol kg}^{-1}$). Carbonate chemistry manipulations were accomplished by first acidifying seawater to an alkalinity of 0 and allowing DIC to offgas. Then alkalinity and DIC were replaced by adding a known amount of a DIC stock (sodium carbonate and sodium bicarbonate) to each incubation vessel and alkalinity adjusted with either HCl or NaOH. Additional incubations further modified salinity and calcium concentrations by the addition of known amounts of distilled water and calcium chloride.

Mussel response was quantified as the calcification rate measured with the alkalinity anomaly technique corrected for ammonia production (Gazeau et al 2015). Calcification rates are expressed as gross calcification where net calcification = gross calcification - dissolution. Dissolution rates were quantified separately and can be found in the dataset: Shell dissolution data for *Mytilus californianus* from March to July 2020 (OA decoupling project). A link to this dataset can be found within the Related Datasets section of this metadata page.

Data Processing Description

We calculated net calcification rates with the ammonia-corrected alkalinity anomaly technique (Gazeau et al 2015), divided by incubation duration and mussel dry tissue mass raised by a factor of 0.72 (see related dataset). The alkalinity anomaly technique builds on the observation that precipitation of CaCO_3 results in an equivalent reduction in seawater $[\text{CO}_3^{2-}]$ (or reduction of $[\text{HCO}_3^-]$ followed by an increase in $[\text{H}^+]$) which contributes two equivalents of total alkalinity—simultaneous production of ammonia is the major metabolic process in mussels that can obscure this and its signal must be removed (Gazeau et al 2015).

Following the incubation, we dissected each mussel and dried it at 60 °C for at least 24 hours to obtain the dry tissue mass (excluding byssal threads) and dry shell mass of each individual mussel. We conducted additional incubations ($n=87$, between 3 and 9 per experiment day) without mussels throughout the trials as experimental blanks to determine background changes in alkalinity (Figure S7). We excluded from our analysis any experimental days where background alkalinity changes exceeded 5 $\mu\text{mol kg}^{-1}$. The mean of the absolute values of alkalinity change during the incubations of these experimental blanks was $1.3 \pm 1.2 \mu\text{mol kg}^{-1}$ ($n = 72$).

We performed all computations with R statistical software, version 4.1.0. We performed carbonate system calculations using the package *seacarb*, using equilibrium constants from Lueker *et al*. We computed linear mixed models using the *lmer* function in the *lmer* package in R and focused on assessing likely candidate parameters as fixed factors, and mussel collection date as a random intercept to account for natural seasonal differences between cohorts. Conditional R^2 was calculated with the package *MuMIn*. We determined parameters for non-linear fits employed to model dissolution rates by minimizing the sum of squares of model residuals using the *optim* function. Colors for plots were chosen from color palettes in the *cmocean* package in R.

BCO-DMO Processing Description

- Removed special characters (e.g., periods) from column names and replaced with underscores
- Converted dates and datetimes from Excel format to %Y-%m-%d and %Y-%m-%dT%H:%M:%S, respectively
- Changed the presentation of species values from "mytilus_californianus" to "Mytilus californianus" and added AphiaID and LSID to the data file
- ph_total and ph_free columns rounded to 3 degrees of precision, and all other float numeric fields rounded to 2 degrees of precision

[[table of contents](#) | [back to top](#)]

Data Files

File
925598_v1_incubation_data_for_Mytilus_californianus_calcification.csv (Comma Separated Values (.csv), 93.96 KB) MD5:7871de9e437a4da1dcd21393967e144e
Primary data file for dataset ID 925598, version 1

[[table of contents](#) | [back to top](#)]

Related Publications

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. doi:10.1016/S0304-4203(00)00022-0 [https://doi.org/10.1016/S0304-4203\(00\)00022-0](https://doi.org/10.1016/S0304-4203(00)00022-0)

Methods

Romanó de Orte, M., Koweek, D. A., Cyronak, T., Takeshita, Y., Griffin, A., Wolfe, K., Szmant, A., Whitehead, R., Albright, R., & Caldeira, K. (2021). Unexpected role of communities colonizing dead coral substrate in the calcification of coral reefs. *Limnology and Oceanography*, 66(5), 1793–1803. Portico. <https://doi.org/10.1002/lno.11722>

Results

[[table of contents](#) | [back to top](#)]

Related Datasets

IsRelatedTo

Gaylord, B. (2024) **Shell dissolution data for Mytilus californianus from March to July 2020 (OA decoupling project)**. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-05-06 doi:10.26008/1912/bco-dmo.925664.1 [\[view at BCO-DMO\]](#)

Relationship Description: Calcification rates are expressed as gross calcification where net calcification = gross calcification - dissolution. Dissolution rates were quantified separately and can be found in this related dataset.

[[table of contents](#) | [back to top](#)]

Parameters

Parameter	Description	Units
species	Mussel species used in incubation.	unitless
AphiaID	Unique identifier for the listed taxon in the Aphia database.	unitless
LSID	Life Science Identifier (LSID) for the listed taxon.	unitless
module	Experimental module corresponding to different mussel collection events.	unitless
date_local	Incubation date in Pacific Standard Time.	unitless
start_datetime_local	Start datetime of the incubation in Pacific Standard Time.	unitless
ISO_Start_DateTime_UTC	Start datetime of the incubation in UTC.	unitless
duration	Duration of incubation in hours.	hours
salinity	Incubation salinity.	PSU
temperature	Incubation Temperature.	degrees Celcius (c)
calcification	Calcification rate of mussel, can be calculated within this dataset as $(-0.5 * (\text{delta.ta} - \text{delta.nh3}) * \text{incubation.water.mass}) - \text{calc.diss} / \text{duration} / \text{tissue.mass}^{0.71592}$.	$\mu\text{mol hr}^{-1} \text{g}^{-0.71592}$
tissue_mass	Dried mussel tissue mass.	grams (g)
shell_mass	Dried mussel shell mass.	grams (g)
wet_mass	Wet mass of mussel prior to incubation.	grams (g)
TA	Mean alkalinity during incubation.	$\mu\text{mol kg}^{-1}$
ph_total	Mean pH during incubation, total scale.	unitless
hco3	Mean bicarbonate ion concentration during incubation.	$\mu\text{mol kg}^{-1}$
co3	Mean carbonate ion concentration during incubation.	$\mu\text{mol kg}^{-1}$
omega	Mean aragonite saturation state during incubation, corrected for calcium concentration where calcium was modified.	unitless
omegac	Mean calcite saturation state during incubation, corrected for calcium concentration where calcium was modified.	unitless
ca	Calcium concentration during incubation, either calculated from salinity or measured with Ca ion selective electrode.	mol kg^{-1}
ph_free	Mean pH during incubation, free scale.	unitless
H	Mean proton concentration during incubation, calculated from ph_free.	unitless
SIR	Mean substrate to inhibitor ratio, $[\text{HCO}_3^-] / [\text{H}^+]$ during incubation.	unitless
co2	Mean carbon dioxide concentration, including dissolved carbon dioxide and carbonic acid concentrations, during incubation.	$\mu\text{mol kg}^{-1}$
pco2	Mean partial pressure of carbon dioxide during incubation.	uatm
dic	Mean dissolved inorganic carbon concentration, $[\text{CO}_2] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]$, during the incubation.	$\mu\text{mol kg}^{-1}$
do	Mean dissolved oxygen concentration during the incubation.	$\mu\text{mol kg}^{-1}$

ci	Mussel condition index, dry tissue mass divided by total dry mass.	umol kg-1
incubation_water_mass	Mass of seawater in incubation vessel.	kilograms (kg)
byssal_threads	Byssal thread production rate during incubation.	threads hr-1
delta_ta	Measured change in alkalinity during incubation.	umol kg-1
delta_nh3	Measured change in ammonia concentration during incubation.	umol kg-1
calc_diss	Correction for alkalinity change due to abiotic dissolution calculated based on shell mass and seawater saturation state, see Figure S2 in related results publication, Romano de Orte et al. (2021).	umol
figure2	Indicates if data were used to generate figures published in related results publication, Romano de Orte et al. (2021). 1= data used to generate Figure 3, 0 = data not used to generate Figure 3.	unitless
figure3	Indicates if data were used to generate figures published in related results publication, Romano de Orte et al. (2021). 1 = data used to generate Figure 4, 0 = data not used to generate Figure 4.	unitless
figure4	Indicates if data were used to generate figures published in related results publication, Romano de Orte et al. (2021). 1 = data used to generate Figure 5, 0 = data not used to generate Figure 5.	unitless
figureS45	Indicates if data were used to generate figures published in related results publication, Romano de Orte et al. (2021). 1=data used to generate Supplementary Figures 4 & 5, 0=data not used to generate Supplementary Figures 4 & 5.	unitless

[[table of contents](#) | [back to top](#)]

Instruments

Dataset-specific Instrument Name	Shimadzu spectrophotometer
Generic Instrument Name	UV Spectrophotometer-Shimadzu
Dataset-specific Description	A Shimadzu spectrophotometer was used to conduct spectrophotometric pH and ammonia analyses.
Generic Instrument Description	The Shimadzu UV Spectrophotometer is manufactured by Shimadzu Scientific Instruments (ssi.shimadzu.com). Shimadzu manufacturers several models of spectrophotometer; refer to dataset for make/model information.

[[table of contents](#) | [back to top](#)]

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 ($[\text{HCO}_3^-]/[\text{H}^+]$), 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.

[[table of contents](#) | [back to top](#)]

Funding

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

[[table of contents](#) | [back to top](#)]