

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

Website: <https://www.bco-dmo.org/dataset/935476>

Data Type: experimental, Other Field Results

Version: 1

Version Date: 2024-12-28

Project

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

Contributors	Affiliation	Role
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Abstract

Reductions to seawater pH challenge the shell integrity of marine calcifiers. Many molluscs have an external organic layer (the periostracum) that limits exposure of underlying shell to the external environment, which could potentially help combat shell dissolution under corrosive seawater conditions. We tested this possibility in adult California mussels, *Mytilus californianus*. We quantified shell dissolution rates as a function of periostracum cover across three levels of reduced pH (7.7, 7.5, and 7.4 on the total scale). This dataset represents shell dissolution data of California Mussels as a function of shell periostracum cover and pH level from lab experiments conducted at the Bodega Marine Laboratory, University of California, Davis in July and August 2022. For the current study, adult mussels (42 - 64 mm in length) were collected from Marshall Gulch, California (38.369738 °N, -123.073921 °W) between August 2021 and March 2022 and transported immediately to the University of California Davis' Bodega Marine Laboratory (< 30 min distance), in Bodega Bay, California. Mussels were held in filtered, flow-through seawater and fed ad libitum until used in experiments. Further experiments on external shell dissolution were performed in the dataset: Lab incubations of mussels (*Mytilus californianus*) examining the influence of simulated abrasion of periostracum on external shell dissolution at Marshall Gulch Beach, CA from August 2021 to March 2022 (see BCO-DMO related dataset).

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Coverage

Location: Marshall Gulch Beach, California

Spatial Extent: Lat:38.369738 Lon:-123.073921
Temporal Extent: 2022-07-20 - 2022-08-24

Methods & Sampling

Overview: Trials examining rates of shell dissolution were conducted in seawater of differing carbonate system conditions, using dead shells of sacrificed adult California *M. californianus* mussel individuals. The relationship between percent surface cover of periostracum and shell dissolution rate under contemporary but chemically stressful seawater conditions was examined. For the current study, adult mussels (42 - 64 mm in length) were collected from Marshall Gulch, California (38.369738 °N, -123.073921 °W) between August 2021 and March 2022 and transported immediately to the University of California Davis' Bodega Marine Laboratory (< 30 min distance), in Bodega Bay, California. Mussels were held in filtered, flow-through seawater and fed *ad libitum* until used in experiments.

Shell preparation and periostracum cover measurements: The extent of intact periostracum coverage was determined for one of two valves of each *M. californianus* mussel. We photographed individual valves with a 12.2-megapixel digital camera (Google Pixel 4a) and then quantified the area of valve covered by periostracum and total valve surface area using ImageJ (software version 1.52a) calibrated to a scale bar. Because we were interested in the effects of the periostracum in protecting the exterior surface of the shell, we sealed the inner, nacre layer of the shell with silicone (Loctite marine silicone sealant) to prevent its contact with seawater.

Seawater manipulation: We used two techniques to establish the different pH treatments employed in our experiments. In the dissolution incubations conducted at pH = 7.5 (n = 49), we modified seawater chemistry using a standard mass-flow control system, bubbling gas of a fixed partial pressure of CO₂ directly into filtered seawater via flow-through sumps. In dissolution trials involving pH = 7.7 (n = 9) and 7.4 (n = 16), we employed an alternative, but equivalent, approach to manipulating seawater pH where we used direct chemical modification to the seawater carbonate system via equimolar additions of 1 M sodium bicarbonate (NaHCO₃) and 1 M hydrochloric acid (HCl). Both methods of seawater manipulation result in an increase of dissolved inorganic carbon (DIC) and reduction of seawater pH without changing total alkalinity (TA).

Dissolution Incubations and Analysis: Following determination of percent cover of periostracum, we incubated each mussel valve in a sealed 250 mL Nalgene bottle filled with modified seawater for 48 hours in a temperature-controlled room, recording seawater properties in each bottle before and at the end of each incubation, including temperature, salinity, and pH (Yellow Springs Instruments Professional Plus Sonde). YSI sonde pH values were calibrated to the total scale based on pH spectrophotometric measurements of m-cresol dye absorbance at the incubation temperature. Incubation bottles were agitated gently once every 8 hours over the course of the incubations to reduce the establishment of strong chemical gradients of their fluid contents. We took discrete seawater samples at the onset and end of each incubation for determination of total alkalinity concentration (TA); these "before-after" measurements of TA enabled quantification of the increase in alkalinity within each bottle over the duration of the incubation, and thereby the rate of dissolution of calcium carbonate (CaCO₃) shell material for individual valves of known periostracum cover. Shell dissolution rates were quantified using standard alkalinity anomaly techniques (analyzing seawater samples in triplicate and selecting median TA for dissolution quantification), which relate calcium carbonate shell loss to an increase in the total alkalinity (TA) of surrounding seawater, per unit time. Across the dissolution trials, control incubations (15% of daily sample size) of modified seawater were conducted to verify minimal background changes in TA.

Data Processing Description

Data analysis: We performed computations with R statistical software, RStudio version 2023.06.2. We performed carbonate system calculations using the package *seacarb*. We analyzed the influence of periostracum cover on CaCO₃ shell dissolution in *M. californianus* adults for trials at pH = 7.5 using a multiple effects linear model where dissolution rate was normalized to shell surface area (mm²) to account for variations in shell size. Normalized dissolution rates were square root transformed to meet linearity requirements for normally distributed residuals. We used backward stepwise model selection using F test significance as the criteria for omitting terms, resulting in a final model that employed both percent periostracum cover and valve length as fixed predictors. Throughout model selection tests, trial date did not significantly explain variability as a random effect so was omitted. Model residual homoscedasticity was verified

using a Breusch-Pagan test (BP = 1.0525, df = 2, p-value = 0.5908), and assumptions of linearity and additivity were assessed visually using model residual and QQ-plots.

Additionally, we used a separate multiple effects linear model across the two additional pH levels (pH = 7.4, 7.7) to assess how seawater pH independently affects patterns of dissolution. Here, we included pH as a numerical fixed predictor, alongside periostracum cover. Due to a constrained sampling size and narrow range of shell lengths (52 mm \pm 4 mm SD), shell length was not included as a fixed predictor in this model in order to conserve degrees of freedom. Normalized CaCO₃ dissolution rate was again square root transformed, and backward stepwise model selection was used to estimate the significance of fixed predictors. A Breusch-Pagan test confirmed residual homoscedasticity (BP = 3.5024, df = 3, p-value = 0.3204), and other assumptions were assessed visually as before.

BCO-DMO Processing Description

1) Processed the submitted file named phdat_PUB.csv with the BCO-DMO tool Laminar.

BCO-DMO parameter naming convention is for names to only include alphanumeric characters and underscores. Periods in the parameter names have been replaced with underscores.

Renamed parameters according to BCO-DMO naming convention and to clarify what the parameter represents.

ph.t.0 renamed to temp_0 to make it clear this is a temperature value and an initial one

shell.L renamed to shell_L following BCO-DMO naming convention

shell.W renamed to shell_W following BCO-DMO naming convention

shell.a.mm2 renamed to shell_A following BCO-DMO convention of removing units from parameter names if possible. Rename it using a capitalized A to match the capitalization of the parameters shell_L and shell_W.

peri.sa renamed to peri_percent to clarify it represents a percent.

incub.hr renamed to incub_hr following BCO-DMO naming convention

incub.min renamed to incub_min following BCO-DMO naming convention

delta.time.days renamed to incub_day to match the naming format of incub_hr and incub_min since they are all incubation durations

alk.t0 renamed to alk_t0 following BCO-DMO naming convention

alk.t1 renamed to alk_t1 BCO-DMO following BCO-DMO naming convention

alk.t0.t1 renamed to alk_t0_t1 BCO-DMO following BCO-DMO naming convention

umol.per.hour renamed to alk_t0_t1_norm to resemble the naming of the change in alkalinity parameter alk_t0_t1 and adding the suffix _norm to indicate it is normalized.

diss.m2 renamed to diss_norm following BCO-DMO convention to remove units from the parameter name, and added the suffix _norm to indicate it is normalized.

Created new parameters time_in_UTC and time_out_UTC by converting local timezone (Pacific Daylight time) parameters time_in and time_out to UTC

Saved dataset to primary data file named 935476_v1_mussel_lab_incubations_periostracum_and_ph.csv

2) Checked species taxonomy of mussel collected, *Mytilus californianus*, and created a taxonomy file using information from the World Register of Marine Species (WoRMS: marinespecies.org) with columns: ScientificName, AphiaID, LSID, Authority, Kingdom, Phylum, Class, Order, Family, Genus, Species

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Data Files

File
935476_v1_mussel_lab_incubations_periostracum_and_ph.csv (Comma Separated Values (.csv), 21.63 KB) MD5:759db5cbf2acbc80409c56eebcc1cf4f
Primary data file for dataset ID 935476, version 1

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Supplemental Files

File
Species taxonomy filename: species_taxonomy.csv (Comma Separated Values (.csv), 240 bytes) MD5:6adb15fd7f2eba705b30b09b7a827689
Species taxonomy from the World Register of Marine Species (WoRMS: marinespecies.org) with columns: ScientificName, AphiaID, LSID, Authority, Kingdom, Phylum, Class, Order, Family, Genus, Species
Column descriptions ScientificName: Genus and Species name AphiaID: Unique taxonomic identifier at the World Register of Marine Species (WoRMS: marinespecies.org) LSID: The Life Sciences Identifier (LSID) is an Interoperable Informatics Infrastructure Consortium (I3C) and OMG Life Sciences Research (LSR) Uniform Resource Name (URN) specification in progress. Authority: The taxonomic authority is the surname of the person who first described the species

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Related Publications

R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>
Software

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. <https://doi.org/10.1038/nmeth.2089>
Software

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Related Datasets

IsRelatedTo

Saley, A., Gaylord, B. (2024) **Field measurements of periostracum cover of mussels (*Mytilus californianus*) from focal population at Marshall Gulch Beach, CA in July and August 2022**. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-12-26 doi:10.26008/1912/bco-dmo.935484.1 [[view at BCO-DMO](#)]

Relationship Description: Mussels from the same population as Dataset 1 & Dataset 2 with field measurements of periostracum cover of mussels

Saley, A., Gaylord, B. (2024) **Lab incubations of mussels (*Mytilus californianus*) examining the influence of simulated abrasion of periostracum on external shell dissolution at Marshall Gulch Beach, CA from August 2021 to March 2022**. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-12-28 doi:10.26008/1912/bco-dmo.935480.1 [[view at BCO-DMO](#)]

Relationship Description: Mussels from the same population as Dataset 1 & Dataset 3 examining the influence of simulated abrasion of periostracum on external shell dissolution

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Parameters

Parameter	Description	Units
INDEX	Shell ID: identification number of individual mussel valve	unitless
species	Mussel species	unitless
lat	latitude, South is negative	decimal degrees
lon	longitude, West is negative	decimal degrees
date_in	Date of incubation start	unitless
time_in	Date and time of incubation start. Local time zone, Pacific Daylight Time	unitless
time_in_UTC	Date and time of incubation start in UTC	unitless
date_out	Date of incubation end	unitless
time_out	Date and time of incubation end. Local time zone, Pacific Daylight Time	unitless
time_out_UTC	Date and time of incubation end in UTC	unitless
ph_spec_0	Initial pH of seawater (total scale) measured by spectrophotometer using m-cresol dye calibration	unitless
temp_0	Initial seawater temperature	Degrees Celsius (°C)
sal_0	Initial seawater salinity	unitless
peri_percent	Periostracum cover: percent cover of intact periostracum over mussel valve	percentage (%)
shell_A	Mussel valve area measurement (ImageJ)	millimeters squared (mm ²)
shell_L	Longest measured length of valve (ImageJ)	millimeter (mm)
shell_W	Widest measured width of mussel valve (ImageJ)	millimeter (mm)

incub_day	Length of incubation in days	day
incub_hr	Length of incubation in hours	hour
incub_min	Length of incubation in minutes	minute
alk_t0	Initial alkalinity: measured alkalinity of seawater prior to incubation	micromole per kg (umol/kg)
alk_t1	Final alkalinity: measured alkalinity of seawater after completion of incubation	micromole per kg (umol/kg)
alk_t0_t1	Alkalinity change: change in alkalinity from initial measurement to final measurement (negative value indicates an increase in alkalinity)	micromole per kg (umol/kg)
alk_t0_t1_norm	Alkalinity change per hour: measured change in alkalinity normalized per hour of incubation	micromole per kg per hour (umol kg ⁻¹ hr ⁻¹)
pH_method	Seawater modification: Method used to modify seawater pH (gas = controlled flow through system; chem = chemical modification)	gas or chem
diss	Dissoluton rate: inverse of alkalinity change measurement	micromole per kg per hour (umol kg ⁻¹ hr ⁻¹)
diss_norm	Dissolution rate by valve area: Dissolution rate normalized to individual shell area measurements	micromole per kg per hour per millimeter squared (umol kg ⁻¹ hr ⁻¹ mm ⁻²)

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Instruments

Dataset-specific Instrument Name	Google Pixel 4a
Generic Instrument Name	Camera
Dataset-specific Description	12.2-megapixel digital camera (Google Pixel 4a)
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Shimadzu spectrophotometer
Generic Instrument Name	UV Spectrophotometer-Shimadzu
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.

Dataset-specific Instrument Name	Yellow Springs Instruments Professional Plus Sonde
Generic Instrument Name	YSI Professional Plus Multi-Parameter Probe
Generic Instrument Description	The YSI Professional Plus handheld multiparameter meter provides for the measurement of a variety of combinations for dissolved oxygen, conductivity, specific conductance, salinity, resistivity, total dissolved solids (TDS), pH, ORP, pH/ORP combination, ammonium (ammonia), nitrate, chloride and temperature. More information from the manufacturer.

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

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Funding

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

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