# Dataset 1: Lab incubations of mussels (Mytilus californianus) in 2022 to examine the influence of periostracum cover and pH on external shell dissolution collected 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**: 2024-08-19

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



| Contributors | Affiliation | Role |
|---|---|---|
| Gaylord, Brian | University of California-Davis (UC Davis-BML) | Principal Investigator |
| Saley, Alisha | University of California-Davis (UC Davis-BML) | Student |
| Merchant, Lynne M. | Woods Hole Oceanographic Institution (WHOI BCO-DMO) | BCO-DMO Data Manager |



**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.

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## Coverage

**Location**: Marshall Gulch, California
**Spatial Extent**: **Lat**:38.369738 **Lon**:-123.073921
**Temporal Extent**: 2021-08-01 - 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 mussel individuals. A first set of experiments determined the relationship between percent surface cover of periostracum and shell dissolution rate under contemporary but chemically stressful seawater conditions. 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 $CO_2$ 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 ($NaHCO_3$) 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 ($CaCO_3$) 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 ± 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.

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## Related Datasets

### IsRelatedTo

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Gaylord, B. (2024) **Dataset 2: Lab incubations of mussels in 2022 to examine the influence of simulated abrasion of periostracum on external shell dissolution collected at Marshall Gulch Beach, CA from August 2021 to March 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-08-19 http://lod.bco-dmo.org/id/dataset/935480 [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*

Gaylord, B. (2024) **Dataset 3: Field measurements of periostracum cover of mussels from focal population collected at Marshall Gulch Beach, CA in July and August 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-08-19 http://lod.bco-dmo.org/id/dataset/935484 [view at BCO-DMO]
*Relationship Description: Mussels from the same population as Dataset 1 & Dataset 2 with field measurements of periostracum cover of mussels*

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| INDEX | Shell ID: identification number of individual mussel valve | unitless |
| species | Mussel species | unitless |
| AphiaID | Unique identifier for the listed taxon in the Aphia database. | unitless |
| LSID | Life Science Identifier (LSID) for the listed taxon. | unitless |
| lat | latitude, South is negative | deicmal degrees |
| lon | longitude, West is negative | decimal degrees |
| date | Date of starting incubation | unitless |
| time_in | Date and time of incubation start | unitless |

| | | |
|---|---|---|
| date_out | Date of end of incubation | unitless |
| time_out | Date and time of incubation end | 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) | milimeters squared (mm^2) |
| shell_L | Longest measured length of valve (ImageJ) | milimeter (mm) |
| shell_W | Widest measured width of mussel valve (ImageJ) | milimeter (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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## 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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