Lab study on the effect of dissolved oxygen, salinity, and temperature on mussel adhesive plaques with mussels collected from Penn Cove Shellfish in Coupeville, Washington.

Website: https://www.bco-dmo.org/dataset/784377
Data Type: experimental
Version: 1
Version Date: 2019-12-13

Project
» Effects of Ocean Acidification on Coastal Organisms: An Ecomaterials Perspective (OA - Ecomaterials Perspective)

Program
» Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Contributors	Affiliation	Role
Carrington, Emily	University of Washington (UW)	Principal Investigator
Haskins, Christina	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Data generated from laboratory experiments that investigated the influence of the dissolved oxygen concentration, salinity, and temperature of seawater on plaque attachment as the material aged. Mussels (M. trossulus) were collected from Penn Cove Shellfish, Quilcene Bay, Quilcene, Washington, USA [47°47’42.0” N, 122°51”10.8” W] and held experimental aquaria at the University of Washington in Seattle, Washington, USA for up to 14 days. Mussels produced threads over the course of 4 hrs that were incubated in a range of dissolved oxygen, temperature, and salinity conditions for up to 12 days. Adhesive plaques were then pulled to failure to determine adhesion strength. These data accompany the manuscript (George, M.N., Pedigo, B., and Carrington, E. 2018).

Table of Contents

Coverage

Spatial Extent: **Lat**:47.795 **Lon**:-122.8527
Temporal Extent: 2015-12 - 2016-02

Dataset Description

Data generated from laboratory experiments that investigated the influence of the dissolved oxygen concentration, salinity, and temperature of seawater on plaque attachment as the material aged. Mussels (*M. trossulus*) were collected from Penn Cove Shellfish, Quilcene Bay, Quilcene, Washington, USA [47°47’42.0” N, 122°51”10.8” W] and held experimental aquaria at the University of Washington in Seattle, Washington, USA for up to 14 days. Mussels produced threads over the course of 4 hrs that were incubated in a range of dissolved oxygen, temperature, and salinity conditions for up to 12 days. Adhesive plaques were then pulled to

failure to determine adhesion strength. These data accompany the manuscript (George, M.N., Pedigo, B., and Carrington, E. 2018).

Methods & Sampling

Adult mussels (Mytilus trossulus, Gould 1850; ~4-6 cm shell length) were collected from aquaculture lines at Penn Cove Shellfish's mussel aquaculture operation located in Quilcene Bay, Quilcene, Washington, USA (47°47'42.0" N, 122°51"10.8" W) during the winter of 2016 (December-February). Mussel were kept in 50 L aquaria for up to two weeks, filled with 0.2 µm filtered seawater and fed Shellfish Diet 1800 (Reed Mariculture, Campbell, CA) up to 5% of wet tissue mass day-1 at an algal concentration of 2000 cells ml-1. Mussels were allowed to attach to mica sheets over the course of 4 hours. Byssal threads were cut away from the animal at the proximal region's interface with the shell. Threads from individuals that made less than three attachments were not included in a treatment group. Mica sheets with plaque attachments were stored dry at room temperature (~21°C, ~30-40% RH) for up to two weeks and then moved into treatment conditions.

Mussels were secured to mica plates with rubber bands and allowed to produce byssal threads for up to four hours in typical open-ocean seawater conditions (pH ~ 8.1, T ~ 10°C, Sal ~ 31 PSU, O2 ~ 8 mg L-1), after which threads were cut away from the animal in the proximal region of the thread (at the shell margin). Only mussels that produced three or more attachments were included in a treatment group. A subset of threads was tested immediately, serving as a 4 hour, 'freshly made' control. The remainder of mica plates with attached threads were placed in one of four treatments ('control', 'oxygen', 'temperature', or 'salinity') and allowed to mature for 12 days, removing a subset of plates at 3, 5, 8, and 12 days in some cases.

Seawater treatments were designed to mimic open-ocean conditions in all ways but one, pushing either temperature, dissolved oxygen, or salinity to the most extreme values seen in estuarine systems that are metabolically driven by the local biota (Lowe et al. 2019). A hypoxia treatment ('oxygen'; O2 <2 mg L-1) was achieved through the injection of N2 gas into a 3 L container, using an aerator. The dissolved oxygen concentration of seawater treatments was monitored in real-time with a DirectLine DL5000 equilibrium probe (accuracy ± 1%) attached to a UDA2182 analyzer (Honeywell, Fort Washington, PA), which controlled the injection of N2 by dynamically opening a solenoid valve in-line with a nitrogen gas cylinder. A high temperature treatment ('temperature'; T = 30°C) was achieved using a 500-Watt titanium aquarium heater and accompanying PID controller (Aquatop Aquatic Supplies, Brea, CA). A low salinity treatment ('salinity'; <1 PSU) was achieved by placing plaques in deionized water. Seawater pH and temperature were monitored in each treatment with a Honeywell Durafet III pH electrode (Martz et al. 2010; accuracy ± 0.01), while salinity was monitored with a DL4000 conductivity cell (accuracy ± 1 PSU).

The adhesion strength of individual plaques was determined by gripping each byssal thread in the distal region, 1 mm away from the adhesive plaque, and pulling perpendicular to the substrate until failure using a tensometer (George and Carrington, 2018). Adhesion strength (kPa) was calculated as the maximum of the force extension curve (N), normalized by the planform area of the attachment plaque measured in mm2 (Burkett et al. 2009). The adhesion strength for 3-5 plaques were averaged and reported as a single value for each mussel. During mechanical testing, the failure mode of each plaque was also visually scored as an adhesive, peeling, or tearing failure, as outlined by Young and Crisp (1982). Adhesive failure occurred when a plaque disengaged from a surface uniformly at the adhesive-substrate interface, while a peeling failure characteristically began at a single point along the outer edge of the plaque, propagating to the rest of the structure. A tearing failure was evident when a portion of the adhesive remained attached to the surface after the test had completed.

The stiffness of the plaque cuticle was determined by following the protocol outlined by George and Carrington (2018). Briefly, stiffness (DMT modulus) was measured using a Dimension ICON atomic force microscope (AFM), fitted with a ScanAsyst-Air probe with a silicon-nitride tip (Bruker, Billerica, MA). Prior to testing, plaques were rinsed with DI water and allowed to dry for 5 minutes. Efforts were taken to probe smooth patches away from the thread-plaque junction, avoiding the innervating roots of the thread. DMT modulus (GPa) was calculated as the slope of the force curve during tip-sample separation. To obtain a representative stiffness of the cuticle, DMT modulus was averaged over a 10 nm2 scan area, with a sampling rate of 512 per line. DMT Modulus was calibrated against a fused silica standard (Veeco, Plainview, NY). Multiple locations (3-5) were scanned for each plaque and then averaged.

In preparation for amino acid (AA) analysis, adhesive plaques were collected from three different seawater treatments (4 hours and 12 days in open-ocean conditions; 12 days in nitrogen infused seawater) and stored in nitrogen flushed microfuge tubes at -80°C for up to 4 weeks. Acid hydrolysis was then performed in vacuo

at 110°C for 48 hours in 6M HCl, with 5% phenol added to preserve DOPA residues. The hydrolysate of each plaque was flash evaporated against DI water and methanol, dissolving the precipitate in 0.02 M HCl. 100 µl of the mixture was then analyzed using an amino acid analyzer system based on ninhydrin-based chemistry (Hitachi L-8900; Tokyo, Japan). A typical spectrum obtained from the analyzer with identified peaks is presented in Figure 6a. The integral of each amino acid peak was divided by the integral of all peaks to determine the relative molar concentration of each amino acid, normalizing against a background of 0.02 M HCl and subtracting the ammonia peak.

Detailed methods and results are provided in George et al., 2018.

Data Processing Description

BCO-DMO Data Manager Processing Notes:

- converted lat/lon listed in the description to decimal degrees for Osprey page.

- added a conventional header with dataset name, PI name, version date

- blank values in this dataset are displayed as "nd" for "no data." nd is the default missing data identifier in the BCO-DMO system.

[table of contents | back to top]

Data Files

File
data2.csv(Comma Separated Values (.csv), 29.13 KB) MD5:cce8e4c56e9c06471def736355788d4e Primary data file for dataset ID 784377

[table of contents | back to top]

Related Publications

Burkett, J. R., Wojtas, J. L., Cloud, J. L., & Wilker, J. J. (2009). A Method for Measuring the Adhesion Strength of Marine Mussels. The Journal of Adhesion, 85(9), 601–615. doi:10.1080/00218460902996903
Methods

George, M. N., & Carrington, E. (2018). Environmental post-processing increases the adhesion strength of mussel byssus adhesive. Biofouling, 34(4), 388–397. doi:10.1080/08927014.2018.1453927
Methods

George, M. N., Pedigo, B., & Carrington, E. (2018). Hypoxia weakens mussel attachment by interrupting DOPA cross-linking during adhesive plaque curing. Journal of The Royal Society Interface, 15(147), 20180489. doi:10.1098/rsif.2018.0489
Results

Lowe, A. T., Bos, J., & Ruesink, J. (2019). Ecosystem metabolism drives pH variability and modulates long-term ocean acidification in the Northeast Pacific coastal ocean. Scientific Reports, 9(1). doi:10.1038/s41598-018-37764-4
Methods

Martz, T. R., Connery, J. G., & Johnson, K. S. (2010). Testing the Honeywell Durafet® for seawater pH applications. Limnology and Oceanography: Methods, 8(5), 172–184. doi:10.4319/lom.2010.8.172
Methods

O'Donnell, M. J., George, M. N., & Carrington, E. (2013). Mussel byssus attachment weakened by ocean acidification. Nature Climate Change, 3(6), 587–590. doi:10.1038/nclimate1846
Methods

Young GA, Crisp D. 1982. Marine animals and adhesion. In: KW Allend Ed Adhes. Vol. 6. England: Barking, Applied Science Publishers, Ltd.; p. 19–39.
Methods

Young, T. J., Monclus, M. A., Burnett, T. L., Broughton, W. R., Ogin, S. L., & Smith, P. A. (2011). The use of the PeakForceTMquantitative nanomechanical mapping AFM-based method for high-resolution Young's modulus measurement of polymers. Measurement Science and Technology, 22(12), 125703. doi:10.1088/0957-0233/22/12/125703
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
exp_group	Indication of whether plaque were part of the control, hypoxia (oxygen), salinity, or temperature experiment.	Unitless
mussel_ID	Mussel sample identifier	Unitless
adhesive_age	Age of adhesive plaque (time after deposition)	Days
temp	Text identifier: high or low treatment	Unitless
oxygen	Text identifier: high or low treatment	Unitless
salinity	Text identifier: high or low treatment	Unitless
shell_length	Length of major shell axis	cm
GI	Gonad Index	Unitless
CI	Condition Index	x10^-3 g cm^-3
failure_mode	Plaque failure mode. 1 = adhesive failure, 2 = peeling failure, 3 = tearing failure	Unitless
plaque_area	Adhesive plaque cross-sectional area	mm^2
max_force	Maximum force required to dislodge plaque	N
adhesion_strength	Maximum adhesion strength required to dislodge plaque	kPa
DMT_loc	Location of DMT modulus measurement, if applicable.	Unitless
DMT_modulus	Stiffness of plaque cuticle as determined by AFM	GPa
Asx	Asparagine or Aspartic in plaque	Mol%
Thr	Threonine in plaque	Mol%
Ser	Serine in plaque	Mol%
Glx	Glutamine or Glutamic acid in plaque	Mol%
Gly	Glycine in plaque	Mol%
Ala	Alanine in plaque	Mol%
Cys	Cysteine in plaque	Mol%
Val	Valine in plaque	Mol%
Met	Methionine in plaque	Mol%
Ile	Isoleucine in plaque	Mol%
Leu	Leucine in plaque	Mol%
DOPA	Dopamine in plaque	Mol%
Tyr	Tyrosine in plaque	Mol%
Phe	Phenylalanine in plaque	Mol%
His	Histidine in plaque	Mol%
Lys	Lysine in plaque	Mol%
Arg	Arginine in plaque	Mol%
Pro	Proline in plaque	Mol%

[table of contents | back to top]

Project Information

Effects of Ocean Acidification on Coastal Organisms: An Ecomaterials Perspective (OA - Ecomaterials Perspective)

Website: http://depts.washington.edu/fhl/oael.html

Coverage: Friday Harbor, WA

Effects of Ocean Acidification on Coastal Organisms: An Ecomaterials Perspective
This award will support researchers based at the University of Washington's Friday Harbor Laboratories. The overall focus of the project is to determine how ocean acidification affects the integrity of biomaterials and how these effects in turn alter interactions among members of marine communities. The research plan emphasizes an ecomaterial approach; a team of biomaterials and ecomechanics experts will apply their unique perspective to detail how different combinations of environmental conditions affect the structural integrity and ecological performance of organisms. The study targets a diversity of ecologically important taxa, including bivalves, snails, crustaceans, and seaweeds, thereby providing insight into the range of possible biological responses to future changes in climate conditions. The proposal will enhance our understanding of the ecological consequences of climate change, a significant societal problem.

Each of the study systems has broader impacts in fields beyond ecomechanics. Engineers are particularly interested in biomaterials and in each system there are materials with commercial potential. The project will integrate research and education by supporting doctoral student dissertation research, providing undergraduate research opportunities via three training programs at FHL, and summer internships for talented high school students, recruited from the FHL Science Outreach Program. The participation of underrepresented groups will be broadened by actively recruiting URM and female students. Results will be disseminated in a variety of forums, including peer-reviewed scientific publications, undergraduate and graduate course material, service learning activities in K-8 classrooms, demonstrations at FHL's annual Open House, and columns for a popular science magazine.

[table of contents | back to top]

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)

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1041213

[table of contents | back to top]