

# Lab study on the effect of seawater pH and aging on mussel adhesive plaques with mussels collected from Penn Cove Shellfish in Coupeville, Washington.

**Website:** <https://www.bco-dmo.org/dataset/783882>

**Data Type:** experimental

**Version:** 1

**Version Date:** 2019-12-10

## 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
<a href="#">Carrington, Emily</a>	University of Washington (UW)	Principal Investigator
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## Abstract

Data generated from laboratory experiments that investigated the influence of seawater pH and byssal thread age on plaque attachment. Mussels (*M. trossulus*) were collected from Penn Cove Shellfish, Penn Cove, Coupeville, Washington, USA (48°13'N 122°42'W) and held in 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 pH conditions for up to 20 days. Adhesive plaques were then pulled to failure to determine adhesion strength. These data accompany the manuscript (George, M.N. and Carrington, E. 2018)

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## Coverage

**Spatial Extent:** Lat:48.2166 Lon:-122.7

**Temporal Extent:** 2015-12 - 2016-02

## Dataset Description

Data generated from laboratory experiments that investigated the influence of seawater pH and byssal thread age on plaque attachment. Mussels (*M. trossulus*) were collected from Penn Cove Shellfish, Penn Cove, Coupeville, Washington, USA (48°13'N 122°42'W) and held in 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 pH conditions for up to 20 days. Adhesive plaques were then pulled to failure to determine adhesion strength. These data accompany the manuscript (George, M.N. and Carrington, E. 2018).

## Methods & Sampling

*Mytilus trossulus* (Gould 1850) were collected during the winter months of December-February, 2015-2016, from Penn Cove Shellfish, located off the coast of Whidbey Island, Washington, USA. Mussels were kept in 50-liter aquaria with recirculating, 0.2  $\mu\text{m}$  filtered seawater (pH = 8.1, T = 10°C, Sal = 31 PSU) for up to two weeks, and were fed Shellfish Diet 1800 (Reed Mariculture, Campbell, CA) up to 5% of wet tissue mass day<sup>-1</sup> at an algal concentration of 2000 cells ml<sup>-1</sup>.

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.

Mica sheets with more than three threads attached were incubated in one of six seawater pH treatments (pHNBS target = 1.0, 3.0, 5.0, 7.0, 8.0, 12.0) and allowed to age for either 0.17, 1, 3, 5, 8, 12, or 20 days. Constant pH treatment levels of pH 3.0-8.0 were achieved by bubbling 3 liter containers of filtered seawater with a dynamically controlled mixture of air and CO<sub>2</sub> gas (O'Donnell et al. 2013). Seawater pH was monitored in each container with a Durafet III pH electrode (Honeywell, Fort Washington, PA; accuracy  $\pm$  0.01), attached to a UDA2182 analyzer that controlled a solenoid valve in line with a CO<sub>2</sub> gas canister.

Treatments were constantly bubbled with air to maintain a dissolved oxygen concentration above 8 mg L<sup>-1</sup>, which was monitored with a Honeywell DirectLine DL5000 equilibrium probe (accuracy  $\pm$  0.1). Salinity was measured daily with a Honeywell DL4000 conductivity cell (accuracy  $\pm$  1 PSU). pH (NBS scale), dissolved oxygen (mg L<sup>-1</sup>), and temperature (°C) were logged every ten minutes. Endpoint pH treatments of pH 1.0 and 12.0 were accomplished through the addition of either 1N phosphoric acid or a mixture of 0.5M potassium hydroxide and 0.5M potassium carbonate. Additions were accomplished using the pH stat system described above with the addition of drip irrigators. These treatments were not intended to accurately mimic the carbonate chemistry regime found in nearshore environments, but rather served to bookend the response curve generated through the manipulation of pCO<sub>2</sub>.

Each individual adhesive plaque was pulled until failure according to the protocol outlined in Bell & Gosline (1996). Plaques were pulled at a 90° angle relative to the substrate to ensure uniformity across samples. For each test, a hemostat was used to grip the distal region, 1 mm above the attachment plaque, and attached to a 10 N digital force gauge (OMEGA, Stamford, CT; accuracy  $\pm$  0.01 N) mounted on a motor-driven testing frame. Extension rate was 10 mm min<sup>-1</sup> and force (N) was recorded at 20 Hz. All threads were rehydrated (>5 mins) in their respective seawater treatment prior to testing. Each plaque was imaged before tensile testing using an AmScope MU1000 camera (Irvine, CA) attached to a dissection microscope.

Plaque attachment area (planform area) in mm<sup>2</sup> was measured using AmScopeX imaging software by tracing the outline of each plaque. Adhesion strength (kPa) was recorded as the maximum force required to remove a plaque from the substrate, normalized by the attachment area (Burkett et al. 2009). Work of adhesion (N m<sup>-1</sup>) was calculated as the area under the force-extension curve (Hamada et al. 2017). The mean of 3-5 plaque pulls was reported for each mussel. The failure mode of each plaque was scored visually as either an adhesive failure (plunger failure), a peeling failure (failure propagated from one side of the plaque to the other), or tearing failure (part of the plaque remained after failure) following the guidelines of Young & Crisp (1982).

Nanoscale surface characteristics of the cuticle of adhesive plaques were determined using a Bruker (Billerica, MA) Dimension ICON atomic force microscope (AFM). A ScanAsyst-Air probe with silicon-nitride tip was used to approach the surface of dry plaque samples, scanning 1  $\mu\text{m}^2$  regions of the cuticle's topography. Scans of the plaque surface were haphazardly taken 1 mm away from the distal root, avoiding large topographical features on the plaque's surface and parts of the distal region that innervated the plaque architecture. A region free of large topographical features was chosen from each 1  $\mu\text{m}^2$  scan for analysis, moving the tip to that section and recording a 10 nm<sup>2</sup> adhesion image. DMT modulus (GPa) was calculated within each scan as the slope of the force curve during tip-sample separation (Young et al. 2011), with a resolution of 512 samples per line and calibrated against a fused silica standard (Veeco, Plainview, NY).

The over 260,000 measurements obtained from each 10 nm<sup>2</sup> scan were averaged to get a representative stiffness of the cuticle at that position. Three samples were taken per plaque, averaging the mean of three plaques from each mussel.

Detailed methods and results are provided in George and Carrington, 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.

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## Data Files

File
<b>dataset_1.csv</b> (Comma Separated Values (.csv), 16.18 KB) MD5:abce4ec73a24b311b1693fe83a2f401f
Primary data file for dataset ID 783882

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## Related Publications

Bell, E.C., Gosline, J.M. (1996). Mechanical design of mussel byssus: material yield enhances attachment strength. *Journal of Experimental Biology* 199(Pt4): 1005–1017.  
*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](https://doi.org/10.1080/08927014.2018.1453927)  
*Methods*

,  
*Results*

Hamada, N., Roman, V., Howell, S., & Wilker, J. (2017). Examining Potential Active Tempering of Adhesive Curing by Marine Mussels. *Biomimetics*, 2(4), 16. doi:[10.3390/biomimetics2030016](https://doi.org/10.3390/biomimetics2030016)  
*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](https://doi.org/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 PeakForce™ quantitative 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](https://doi.org/10.1088/0957-0233/22/12/125703)  
*Methods*

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## Parameters

Parameter	Description	Units
experiment	Exp_1 = plaque aging experiment; Exp_2 = pH maturation experiment	Unitless
mussel_ID	Mussel sample identifier	Unitless
adhesive_age	Age of adhesive plaque (time after deposition)	Days
pH	Seawater pH treatment plaques were aged in (NBS scale)	Unitless
shell_length	Length of major shell axis	cm
failure_mode	Plaque failure mode. 1 = adhesive failure, 2 = peeling failure, 3 = tearing failure	Unitless
GI	Gonad Index	Unitless
CI	Condition Index	$\times 10^{-3} \text{ g cm}^{-3}$
plaque_area	Adhesive plaque cross-sectional area	$\text{mm}^2$
max_force	Maximum force required to dislodge plaque	N
adhesion_strength	Maximum adhesion strength required to dislodge plaque	kPa
work_adhesion	Work of adhesion	$\text{N m}^{-1}$
DMT_modulus	Stiffness of plaque cuticle	GPa

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## 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.

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## 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](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](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 \(NSF\)](#)

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1041213</a>

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