Lab study on the effect of temperature and pCO2 on mussel byssal attachment (thread mechanics) with mussels collected in May 2012 from Argyle Creek, San Juan Island, WA (48.52˚ N, 123.01˚ W)

Website: https://www.bco-dmo.org/dataset/773556
Data Type: experimental
Version: 1
Version Date: 2019-07-24

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
Soenen, Karen	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
These data were used in a structural analysis study to evaluate how pCO2 and an additional stressor, elevated temperature, influences byssal thread quality and production. Mussels (M. trossulus) were collected in May 2012 from Argyle Creek, San Juan Island, WA (48.52˚ N, 123.01˚ W) and held in a mesh box submerged under the dock at Friday Harbor Laboratories (FHL), San Juan Island, WA for up to 14 d. Mussels were placed in controlled temperature and pCO2 treatments in the Ocean Acidification Experimental Laboratory (OAEL), then newly produced threads were counted and pulled to failure to determine byssus strength.

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Coverage

Spatial Extent: **Lat**:48.52 **Lon**:-123.01
Temporal Extent: 2012-05 - 2012-05

Dataset Description

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then newly produced threads were counted and pulled to failure to determine byssus strength.

Methods & Sampling

Mussels (*M. trossulus*) were collected in May 2012 from Argyle Creek, San Juan Island, WA (48.52˚ N, 123.01˚ W) and held in a mesh box submerged under the dock at Friday Harbor Laboratories (FHL), San Juan Island, WA for up to 14 d. Mussels were placed in experimental mesocosms in the Ocean Acidification Experimental Laboratory (OAEL) at FHL as described in O'Donnell et al. (2013) and Timmins-Schiffman et al. (2012). Briefly, manipulations of pH were made by bubbling CO2 into a 150 L temperature-controlled seawater reservoir, that supplied water to eight 3.5 L chambers at a turnover rate of 50 ml min-1. Air was bubbled into the reservoir to maintain 100% oxygen saturation and submersible pumps (model number P396, Annex Depot, Sacramento, CA) provided mixing in the chambers at 3.8 L min-1. The bottom of each chamber was lined with autoclaved pebbles, collected from an FHL beach, to provide a substrate for byssal thread attachment. pH and temperature were monitored continuously in each water reservoir with a Durafet pH and temperature probe and the full carbonate chemistry of the system evaluated with DIC and Total alkalinity measurements once during each trial. Mussels were acclimated to their treatment temperatures in ambient pH (~7.8) over 9 d, ramping temperature up no more than 2˚C per day, and fed a maintenance level of Shellfish Diet 1800 (6 g l-1 day-1, Reed Mariculture, Campell, CA, USA).

The twelve independent temperature x pCO2 treatments spanned the range of local marine conditions (Newcomb, 2015; George *et al.*, 2019; temperature at 10˚C, 18˚C, or 25˚C and pCO2 at 400, 750, 1200, or 2500 µatm). Each mussel was trimmed of external byssus before placement in an experimental treatment for 3 d, sufficient time to produce new mature byssal threads (Bell & Gosline 1996) while minimizing the effect of treatment on mussel condition. Mussels were starved during the 3 d trials to minimize changes in chamber water chemistry due to food addition and to reduce fouling. Three trials were conducted in succession to replicate treatments over time, increasing sample size (n=8 x 3) for each temperature*pCO2 treatment.

At the end of each trial, mussels and the rocks to which they had attached with byssal threads were removed from the chambers. The entire byssus was dissected from each mussel and stored air-dried for up to 20 days. Byssus was rehydrated in seawater prior to testing, a method that does not alter the mechanical properties of the byssal threads (Brazee, 2004). The number of byssal threads each mussel produced was counted, and one thread was haphazardly chosen for mechanical testing following the procedure of Bell & Gosline (1996). Briefly, an individual thread was clamped with submersible pneumatic grips on either end by holding the proximal byssal stem between cardstock with cyanoacrylate glue and affixing the distal plaque with attached rock to an aluminum T-bar with epoxy. An Instron 5565 tensometer (Norwood MA, USA), extended the thread at a rate of 10 mm min-1 in a temperature-controlled water bath (3130-100 BioPuls Bath, Instron, Norwood, MA, USA) until failure. The tensometer measured force (±10-3 N) and extension (± 10-3 mm) at 10 Hz. Tests were performed in seawater with a pH of 7.8 and the relevant treatment temperature.

Pull to failure mechanical tests provided estimates of thread breaking force, yield force, extensibility, initial stiffness and failure location (Bell & Gosline 1996). Yield, due to quasi-plastic deformation in the distal region, was identified as the point where the initial slope of the force-extension curve decreased by 40%. Extensibility was calculated by dividing thread extension at failure by initial length and initial stiffness was determined from the initial slope of the force extension curve. The location of failure (proximal, plaque, and/or distal region) was noted and threads were retested to quantify the breaking force of each remaining region. Tests that broke at the grips were considered underestimates and were discarded.

The cross-sectional area of the proximal region was measured to evaluate morphological differences among treatments. The elliptical area was estimated from measures of the major and minor axes (+ 1 um using a dissecting microscope (Brazee & Carrington 2006). Proximal breaking stress (N mm-2), a material property, was calculated as proximal breaking force divided by proximal area. Thread surface structure was examined using a scanning electron microscope (FEI Sirion XL30 SEM, Hillsboro, OR).

Whole mussel attachment strength was estimated using two mathematical models developed by Bell & Gosline (1996). Each model assumes a mussel is anchored with a constant thread number (n=50) arranged in a circle. The normal model estimates dislodgment force perpendicular to the substrate (e.g, lift); all threads are engaged and extend until they reach their maximum force. The parallel model estimates dislodgement force for an animal pulled parallel to the substrate (e.g., drag); threads on the upstream side are the first in tension, yield and extend until they reach maximum force and break, while more threads are recruited into tension until they have all broken. Additionally, we modified each model to incorporate the variation in thread production across treatments. Because thread production was measured for only three days, treatment means were scaled to a

maximum value of 50 threads.

Detailed methods and results are provided in Newcomb, 2015 and Newcomb *et al.,* 2019

Location: Friday Harbor Laboratories, Friday Harbor WA

Data Processing Description

BCO-DMO processing notes:

- Renaming column headers

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Data Files

File
thread_mechanics.csv (Comma Separated Values (.csv), 23.51 KB) MD5:8c7f487a73df506aebb1b0b521276936 Primary data file for dataset ID 773556

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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

Brazee, S. L., & Carrington, E. (2006). Interspecific Comparison of the Mechanical Properties of Mussel Byssus. The Biological Bulletin, 211(3), 263–274. doi:10.2307/4134548
Methods

Brazee, S.L. (2004). An interspecific comparison of biomechanical properties and morphometrics of mussel byssal threads. MS thesis, University of Rhode Island. 52 p.
Methods

George, M. N., Andino, J., Huie, J., & Carrington, E. (2019). Microscale pH and Dissolved Oxygen Fluctuations within Mussel Aggregations and Their Implications for Mussel Attachment and Raft Aquaculture. Journal of Shellfish Research, 38(3), 795. https://doi.org/10.2983/035.038.0329
Methods

Newcomb, L. A., George, M. N., O'Donnell, M. J., & Carrington, E. (2019). Only as strong as the weakest link: structural analysis of the combined effects of elevated temperature and pCO2 on mussel attachment. Conservation Physiology, 7(1). https://doi.org/10.1093/conphys/coz068
Results

Newcomb, L.A. (2015). Elevated temperature and ocean acidification alter mechanics of mussel attachment (doctoral dissertation), University of Washington, Washington, USA
Results

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

Timmins-Schiffman, E., O'Donnell, M. J., Friedman, C. S., & Roberts, S. B. (2012). Elevated pCO2 causes developmental delay in early larval Pacific oysters, Crassostrea gigas. Marine Biology, 160(8), 1973–1982. doi:10.1007/s00227-012-2055-x
Methods

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Parameters

Parameter	Description	Units
Mussel_ID	Mussel sample identifier	unitless
Temp	Temperature	degrees Celcius (°C)
pH	pH (total scale)	unitless
pCO2_Target	Target pCO2 level	microatmosphere (uatm)
Treatment_ID	Treatment identifier	unitless
Trial	Trial identifier	unitless
GI	Gonad Index	unitless
CI	Condition Index	gram per cubic millimeter (g/mm^3)
Distal_Diam	Thread distal region diameter	millimeter (mm)
Prox_Major	Thread proximal region major axis length	millimeter (mm)
Prox_Minor	Thread proximal region minor axis length	millimeter (mm)
Yield	Thread yield force	Newton (N)
Location	Thread failure location	unitless
Breaking_Ext	Thread breaking extension	millimeter (mm)
Breaking_Force	Thread breaking force	Newton (N)
Plaque_Force	Thread plaque breaking force	Newton (N)
Proximal_Force	Thread proximal region breaking force	Newton (N)
Distal_Force	Thread distal region breaking force	Newton (N)
Breaking_Stress	Thread breaking strength	Newton per square millimeter (N/mm^2)
Youngs_Mod	Thread Youngs modulus	Newton per square millimeter (N/mm^2)
Thread_Stiffness	Thread stiffness	Newton per square millimeter (N/mm^2)
Thread_Ext	Thread extensibility	unitless

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Instruments

Dataset-specific Instrument Name	Instron 5565 load frame
Generic Instrument Name	Materials Testing System
Dataset-specific Description	Instron’s (Norwood, MA) electromechanical testing systems are used to test a wide range of materials in tension or compression. The series 5560 are dual column table top models, the 5565 model has a load capacity of 5 kN (1125 lbf).
Generic Instrument Description	Testing systems that are used to test a wide range of materials in tension or compression.

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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

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)

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Funding

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

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