Year 3 Progress Report
February 1, 2016 – January 31, 2017

Impacts of ocean acidification on wild and farmed mussels in Puget Sound, WA

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Project Goal: We hypothesize that high temperature and ocean acidification are key stressors to mussels, and that these external stressors represent a significant energetic demand that can be modulated by food supply and seasonal spawning cycles. Mussel species that differ in reproductive timing (e.g., the native spring spawner *Mytilus trossulus*, the introduced winter spawner *M. galloprovincialis* and the continuous spawner *M. californianus*) will have different responses to environmental perturbations. The project will link laboratory studies and field observations to test for causes of seasonal weakening of mussel attachment, which in turn causes substantial mortality due to fall-off (also known as "dislodgment" or "sloughing"). Our results are important in identifying the mechanisms by which natural mussel distributions and aquaculture yields are dependent on local changes in environmental conditions, of either natural or anthropogenic origin.

Our four specific objectives, with our activities during the reporting year, are presented here:

1) **Examine the influence of food supply and reproductive state on the independent and combined effects of OA and temperature on mussel attachment in native and naturalized mussel assemblages, identifying the physiological and environmental conditions that lead to mussel fall-off.**
   Progress in Year 3:
   a. Laura Newcomb submitted two manuscripts on her laboratory manipulations, both currently in review. One manuscript (for *Functional Ecology*) describes the combined effects of two stressors, low pH and high temperature, on byssal thread strength. Both stressors are harmful but interestingly, when in combination the harmful effects are antagonistic and not additive or synergistic. The second manuscript (Marine Ecology Progress Series) explored in detail the effects of increased temperature on the byssus (both thread quantity and quality), showing opposite responses to warming above above 20°C by the native *M. trossulus* versus the nonnative *M. galloprovincialis*. The implication for future warmer climates is a competitive advantage (stronger attachment) for the nonnative species, *M. galloprovincialis*.
   b. Molly Roberts (graduate student, also with NSF IGERT funding) ran a laboratory experiment in the laboratory manipulating seawater temperature and food availability over 3 months, and then measured survival, growth, thread production and thread strength. She learned that there were species-specific differences in the effects of temperature on food on growth versus thread production. These results suggest that in optimal conditions energy may be prioritized towards producing

byssal threads over growth. Molly will incorporate these data into her energy budget models and is preparing a manuscript for submission in Autumn 2017.

c. Matt George (graduate student, also with NSF GRF and UW RRF funding) has conducted a series of lab studies on the effects of fluctuating pH conditions on mussel byssus adhesive. His data show newly formed byssal threads require exposure to >pH 6 to 'trigger' the tanning (strengthening) process. Threads that remain in low pH water never strengthen. Moreover, threads that were tanned at high pH gradually weaken when placed in low pH water. Altogether, these data illustrate that the strength of a given thread after it has been molded and released by the mussel, can be dynamically altered by water conditions that change over the course of days to weeks. We see fluctuations of this magnitude and time scale at Penn Cove (see objective 2). These manuscripts are in the early stages of development and should be submitted by the end of 2017.

d. Lyda Harris (graduate student) ran pilot experiments at FHL manipulating microplastic type, size, and quantity on mussel survival and filtration rate. She learned mussels ingest microplastics of varying sizes in all concentrations tested. These pilot studies will inform her dissertation work, on the effects of microplastic on mussel feeding behavior, energetics and long term survival.

2) **Quantify the environmental conditions native and naturalized mussels encounter in wild and farmed habitats, at both the macro and microhabitat scale (e.g., near the mussel where their attachment structures are formed).**

Progress to date:

a. Partnering with Penn Cove Shellfish, we have deployed two YSI data sondes near mussel rafts in Penn Cove, WA (*M. trossulus*). We partnered with NANOOS (NOAA) to live-stream these data to a public visualization platform. We now have nearly three years of monitoring (since August 2014) that indicates threshold values known to weaken byssus (pH < 7.6, temp > 20°C) are exceeded for periods of up to two weeks. We also see that low pH events are coupled with reduced DO and, in some cases, hypoxia. In addition, seasonal freshening events reduce surface salinity and often co-occur with warming in summer and cooling in winter. This work was led by Hilary Hayford (post-doc) and Molly Roberts.

b. We expanded our observation network to Quilcene Bay WA, a site where Penn Cove Shellfish grows *M. galloprovincialis*, partnering with the WA Department of Natural Resources. WA-DNR provided funds to purchase two more YSI data sondes for this site have been live-streamed to NANOOS for over one year. This work was led by Matt George.

c. These data will be incorporated into several publications (see objective 3).

d. Matt George made several short-term measurements of pH conditions within mussel aggregations, showing substantially lower pH (up to 3.0 units lower!) than nearby 'open water' conditions captured by our data sondes. This indicates the microclimate at the scale where mussels produce new byssal threads can be very different than what we measure at a larger scale.

e. Molly Roberts manipulated mussels in the field by placing them at different depths, and moving them to experimental enclosures every 1-2 months for a two week duration over the course of a year (4/2016 - 1/2017) to measure growth,

attachment, and survival in these enclosures. Results are still being worked up but she learned that there was high mortality of the local *M. trossulus* species in shallow water in the early summer, and there was high mortality of *M. trossulus* at depth in the late summer.

f. Due to time and budgetary constraints, we have not monitored conditions for wild mussel populations.

3) Assess the field performance and retention of mussels in both natural and farmed populations.

Progress to date:

a. We now have over three years of monthly mussel attachment and condition measurements at Penn Cove (Laura Newcomb, Molly Roberts, Hilary Hayford). Mussels show a strong seasonal cycle in attachment strength (especially in surface waters) and preliminary analyses suggest water warming and pH are the best predictors of weak attachment. We are currently updating these analyses to include the full three year dataset and a manuscript will be submitted in September 2017.

b. We now have over two years of similar data at Quilcene Bay (Penn Cove Shellfish) and Totten Inlet (Taylor Shellfish). Preliminary analyses show mussel attachment at these two sites is overall weaker than in Penn Cove and that there is no seasonal trend in these data.

c. A new direction for this project was the analysis of annual trends in shell thickness. Hilary Hayford supervised undergraduate interns measuring of shell thickness and erosion of Penn Cove mussels from vouchers collected over past 3 yrs. These data suggest that decreased shell thickness occurs in summer and may be initiated internally and not due to external erosion. Data will be compared to two years of monthly data previously taken from Friday Harbor.

4) Outreach—Develop a high school chemistry curriculum and summer field camp curriculum on ocean acidification.

Progress in Year 3:

a. For the OA chemistry curriculum, we tested several hand-held meters for use in measuring pH and use in alkalinity titrations. One meter was far superior to the others and is being used in the development of the class exercise. A safe pH indicator and CO_2 delivery methods were also selected. We have expanded our approach from an alkalinity focused curriculum to include a broader demonstration of overall ocean chemistry. A power point presentation is in the process of completion. We anticipate having a test ready curriculum this summer.

b. We have engaged the scientific community and general public through scientific presentations, public talks and several media outlets. These activities are outlined in the Project Documents and Publications section of this report.

c. FHL Open House. In May 2016, we showcased our science research and educational activities to over 600 people at the Friday Harbor Laboratories open house (Hilary Hayford, Molly Roberts, Lyda Harris).

d. SGA Congressional Briefing Sept 21, 2016. *Economic Resiliency in the Nation's Coastal Communities: A Briefing Sponsored by the Sea Grant Association in*

cooperation with the Senate Commerce, Science, and Transportation Committee. Ian Jefferds, Owner and General Manager of Penn Cove Shellfish from Washington gave a presentation on the impacts of ocean acidification on wild and farmed mussels and their economic implications. 70 participants.

UW Undergraduate Interns/Volunteers:
MacKenzie Edelsward, Rachel Boccamazzo, Jonathan Huie, Benjamin Makhlouf, Jessie Andino, Lynn Madd, Nadia Ahmed, Hailey Murray, Windy Madden

Project Documents and Publications

Publications:

Newcomb, L.A., M.N. George, M.J. O'Donnell, and E. Carrington. A mechanism for antagonistic interaction between two stressors: effects of temperature and pCO2 on mussel attachment, in review.

Newcomb, L.A., and E. Carrington. The attachment strength of congener mussel species responds in opposite directions to elevated temperature, submitted.

Presentations:

Carrington, E. *How Ocean Acidification and Ocean Warming Could Influence the Functional Morphology and Ecology of Intertidal Organisms*, a symposium in the 2016 AAAS Pacific Division Annual Meeting, June 14-17, 2016. Title: "Hanging on by a Thread? Mussel Attachment in a Warmer, Higher CO_2 World." Invited speaker, 60 participants.

Carrington, E. *Mollusks in Peril*, a forum hosted by the Bailey-Matthews National Shell Museum on Sanibel Island, May 22-24, 2016. Title: "Hanging on by a Thread? Mussel Attachment in a Warmer, Higher CO_2 World." Invited speaker, 70 participants.

Carrington, E., M. George, H Hayford, L. Newcomb, C. Friedman and I. Jefferds, 2017. All washed up? Mussel survival in the face of ocean warming and acidification. Annual meeting of the Society for Integrative and Comparative Biology, New Orleans, LA. January 4-8. 120 participants.

Carrington, E., M. George, L. Newcomb, C. Friedman, I. Jefferds and M. O'Donnell, 2016. Losing their lifeline? The effects of ocean warming and acidification on mussel attachment. Annual Meeting of the Society of Experimental Biology, Brighton, England. July 3-7. 100 participants.

Carrington, E. Several invited seminars were given at academic institutions featuring this project:

- Departmental Seminar, University of Maine, Orono ME and Darling Marine Center Walpole, ME, December 5-7 2016. Hosted by Maine Sea Grant. 50 participants
- Departmental Seminar, Virginia Institute for Marine Science, Glouster VA, November 30, 2016. 50 participants

George, MN and E. Carrington. The ecomechanics of mussel attachment. Salish Sea Ecosystem Conference, April 12-15, 2016. The Westin Bayshore, Vancouver, B.C., Canada. 100

participants.

George, M.N. and E. Carrington, 2017. Mussels use seawater pH as a molecular trigger in the formation of byssus adhesive. Annual meeting of the Society for Integrative and Comparative Biology, New Orleans, LA. January 4-8. 70 participants.

Newcomb L.A., Jefferds, I, Friedman, C.S., Carrington, E. (2016). Seasonal weakening of *Mytilus trossulus* attachment strength can be predicted by high temperature, low pH, and low dissolved oxygen. Gordon Research Conference on Ocean Global Change Biology, USA. July 17-22, 2016 Waterville Valley, NH. (poster) 50 in audience, $1850 from Knauss Fellowship Professional Development funds

Newcomb L.A., Jefferds, I, Friedman, C.S., Carrington, E. (2016). Seasonal weakening of *Mytilus trossulus* attachment strength can be predicted by high temperature, low pH, and low dissolved oxygen. Benthic Ecology Meeting, Portland, ME, USA. (talk) March 16-19, 2016. 60 in audience, $1080 from Knauss Fellowship Professional Development funds

Harris, Lyda and E. Carrington, UW Graduate Climate Conference, Pack Forest, Poster-- Impacts of microplastic and ocean warming on mussels in the Pacific Northwest, October 28-30, 2016. ~75 people

Benjamin Pedigo (presenter) Matthew George, MacKenzie Edelsward, and Emily Carrington. Environmental Conditions Influence the Formation and Function of Mussel Byssus Adhesive. The Undergraduate Research Symposium, May 20, 2016, Mary Gates Hall, University of Washington, Seattle, WA, USA. Audience: ~300.

Open House, UW Friday Harbor Laboratories. May 15, 2016. Hilary Hayford and Lyda Harris developed a hands-on demonstration of the effects of ocean acidification on shellfish, especially mussels. 200 Attendees.

Media Publications featuring our project:

UW Today, 2016. Acid attack – can mussels hang on for much longer? July 6, 2016. http://www.washington.edu/news/2016/07/06/acid-attack-can-mussels-hang-on-for-much-longer/

The Early Edition, 2016. Live radio interview with Rick Cluff on CBC/British Columbia radio. July 6, 2106.

Johnson, L. 2016. Ocean acidification will make it hard for mussel to hang on experiments suggest. CBC News British Columbua July 6, 2016. http://www.cbc.ca/news/canada/british-columbia/ocean-acidification-will-make-it-hard-for-mussels-to-hang-on-experiments-suggest-1.3666017

Bodkin, Henry, 2016. Mussels off the menu within 85 years due to climate change. Feature story in *The Telegraph* (London, UK) Science edition, July 6, 2016. http://www.telegraph.co.uk/science/2016/07/06/mussels-off-the-menu-within-85-years-due-to-climate-change/.