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

Emily Carrington[1] and Carolyn Friedman[2]
[1]Friday Harbor Marine Laboratory, University of Washington, Friday Harbor, WA
[2]School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA

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 to date, 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.**
    a. Laura Newcomb (doctoral student, degree conferred in 2015) recently resubmitted two manuscripts on her laboratory manipulations to the Journal of Experimental Biology. One manuscript 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 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*. Laura also just submitted a third manuscript to the Journal of Shellfish Research, in which three years of sampling at Penn Cove shows mussel attachment varies seasonally (weakening in summer) and is best predicted by fluctuations in temperature, dissolved oxygen and low pH.
    b. Molly Roberts (doctoral student) has presented her research at several venues, and will be submitting her first manuscript later this year. She conducted a laboratory experiment 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 and food on growth versus thread production. These results suggest that in optimal conditions energy may be prioritized towards producing byssal threads over growth.

c. Matt George conducted a series of lab studies on the effects of fluctuating pH and hypoxic 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. Similarly, low dissolved oxygen hinders compromises adhesive strength and this effect is reversible when beneficial conditions are restored. Low salinity or high temperature, had no effect on the adhesive. 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 (specifically, low pH and low DO) that change over the course of days to weeks. We see fluctuations of this magnitude and time scale at Quilcene and Penn Cove (see objective 2). One manuscript was published in March 2018 and two others will be submitted in May 2018.

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).**

a. We partnered with Penn Cove Shellfish to deploy two YSI data sondes near mussel rafts in Penn Cove, WA (*M. trossulus*). We worked with NANOOS (NOAA) to live-stream these data to a public visualization platform. We now have nearly four 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.

b. We also collected similar environmental data at 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 the two more YSI data sondes for this site have been live-streamed to NANOOS for over one year.

c. Matt George deployed pH/O2 sensor in mussel aggregations, illustrating how the microenvironment where mussels attach can be drastically different than the 'ambient' mussel raft conditions just 1 m away. This is due to a combination of reduced flushing and increase metabolism by mussels and the biological community they support.

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

e. Due to time and budgetary constraints, we did not monitor conditions for wild mussel populations.

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

a. We now have over three years of monthly mussel attachment and condition measurements at Penn Cove (Laura Newcomb, Molly Roberts, Hilary Hayford) and over two years of similar data at Quilcene Bay (Penn Cove Shellfish) and Totten Inlet (Taylor Shellfish). At Penn Cove, 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. At Quilcene and Totton Inlet, mussels are overall weaker than in Penn Cove and that there is no seasonal trend. These have been incorporated into a publication led by Laura Newcomb that will be submitted to the Journal of Shellfish Research in May 2018.
b. Molly Roberts continues to measure growth, attachment, and survival of mussels in enclosures. She has also initiated video transects of mussel abundance which will be used to quantify the magnitude and timing of fall-off events. These data are still being worked up for her dissertation and a manuscript will be likely submitted in 2019.
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4) Outreach—Develop a high school chemistry curriculum and summer field camp curriculum on ocean acidification.

a. Led by the efforts of Carolyn Friedman, we developed a new ocean acidification chemistry curriculum. We decided to change the lesson plan design to add the combination of examining the effects of differing amounts of CO_2 and at different salinities. Dr. Friedman's technician who was working on this is now working in another lab and he was not able to finish updating all of these changes, but they will be added in the coming months. The current versions of these materials are uploaded as Additional Documents.
b. The findings of this project inspired the development of an educational video, "Hanging by a Thread - Mussels in a Changing Ocean" by Abby Lunstrum and Laura Newcomb. This stop-motion animation shows how climate change alters ocean temperature and chemistry, and explains the potential impacts on one example species: the blue mussel. The URL for the video is: https://www.youtube.com/watch?v=ew3cGFiNbs0&feature=youtu.be. The video was presented by Meg Chadsey at the Ocean Sciences Meeting in February 2018.
c. We have engaged the scientific community and general public through numerous scientific presentations, public talks and several media outlets. These activities are outlined in the Project Documents and Publications section of this report.
d. The major findings of this research have also been presented to a number of governmental stakeholders, including Washington State legislators (Gov. Jay Inslee, visit to our field site) and United States Congressional staffers (SGA Congressional Briefing on Economic Resiliency in the Nation's Coastal Communities) as well as university liaisons (e.g., Association for Public and Land Grant Universities).