Changes in the proteome of mussel feet (*Mytilus trossulus*) in response to elevated pCO_2

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Abstract

Experiment is currently in progress. Will have data to analyze on Monday, 12/2.

1. Introduction

Mussels (*Mytilus sp.*) play a key role in the formation of biological communities in the rocky intertidal zone. Aggregating into groups, mussels form beds which prevent the erosion of coastlines (Meadows et al., 1998), provide habitat for smaller organisms (Suchanek, 1979), and improve water quality through filter feeding (Lindhl et al., 2005). Mussels are also an aquaculture species, supporting a worldwide industry worth more than $1.5 billion annually (FAO, 2011). However, despite their environmental and commercial significance, little is known about how the effects of climate change, such as the rapid uptake of CO_2 into the oceans (ocean acidification), will impact the physiological ecology of these organisms.

What makes mussels unique and an amenable aquaculture species is their ability to attach to rocks and aquaculture lines. Mussels accomplish this by anchoring themselves to surfaces using byssal threads laced with a natural adhesive. Synthesized in seawater, curing within minutes, and able to selfheal after fatigue, byssal threads are a biomechanical marvel which have inspired the synthesis of several novel synthetic glues (Lee et al., 2011).

Despite the importance of byssal threads, to date, studies investigating the effect of ocean acidification (OA) on mussels have mainly been concerned with the calcification rate of shell material (Gazeau et al., 2007) and larval development (Kurihara, 2008). In fact, O'Donnell et al. (2013), published this year, is currently the only study to investigate the sensitivity of byssal thread synthesis to environmental factors which are changing as a result of ocean acidification. O'Donnell et al. (2013) found that byssal threads produced under elevated pCO_2 conditions were significantly weaker than those produced under ambient condition; a result, which the authors applied to an attachment model from Bell and Gosline (1996), predicts an increase in dislodgment events of up to 40% as a result of ocean acidication.

A mechanistic explanation for thread weakening as a result of ocean acidification has yet to be determined. One possibility is that the threads themselves become less stiff under acidified conditions. Weakening of the thread itself, however, has only been seen in lab experiments were naturally grown threads are exposed to acidic buffer solutions (Harrington et al., 2007) and was not evident in threads produced in water with elevated pCO_2 (O'Donnell et al., 2013). A more plausible explanation is that byssal thread attachment at the thread-substrate interface is impacted by water chemistry in some way.

Byssal threads attach to substrates using an adhesive made up of ten identified proteins, several of which are unique to foot tissue (Hwang et al., 2011). Of the ten, several proteins contain high concentrations of DOPA, a molecule known for its pH dependent adhesive properties. As a result, studies investigating byssal thread adhesion up to this point have taken a single protein focus, often isolating a protein target and allowing it to bind to a target surface under certain conditions (Lee et al., 2006). What this approach ignores is the potential shift in protein expression that can come with environmental changes. To take this into account, I exposed mussels to two pCO_2 treatments and harvested protein samples from foot tissue after a two week acclimation period. Using proteomic techniques I was then able to look for shifts in the quantity and presence of the proteins involved in adhesion.

2. Materials and Methods

2.1. Organism care and seawater manipulation

Mussels (*Mytilus trossolus*) were collected from field sites near Alki Beach, Seattle, WA, USA (+47.58, -122.40) and brought into a recirculating seawater laboratory at the University of Washington, Seattle, WA. Ten individuals were split between two 50 gallon seawater tanks set to pCO_2 concentration reflecting ambient (~700 µatm) and elevated (~1800 µatm) conditions. Desired CO_2 concentrations were obtained by bubbling a mixture of ambient air and CO_2 into each tank at a constant rate (1.5 L/min air : 1.5 ml/min CO_2), allowing the water volume to equilibrate, and measuring accuracy using a pH probe every few days (see Table 1). Samples of food tissue were harvested from each mussel and immediately frozen at -80°C. Two individuals were sampled before the experiment began to establish a baseline while the rest of the mussels were sampled after remaining in the seawater treatments for two weeks.

2.2. Tissue homogenization

The ZOOM® IPGRunner™ combo kit was used to analyze protein samples. 50 mg of foot tissue was thawed and homogenized using a plastic pestle and 950 µl of a lysis buffer consisting of 1.1X ZOOM® 2D solubilizer 1 solution (ZS10001), 1M Tris Base, 100X protease inhibitor cocktail (Roche catolg no. 1873580), 2M DTT, and DI water as outlined by the ZOOM® IPGRunner™ protocol. Lysate was incubated on a rotary shaker for 15 minutes at room temperature after which 5 µl of N,N-Dimethylacrylamide (DMA) was added to each sample and allowed to alkylanate the sample for 30 minutes at room temperature. 10 µl of 2M DTT was then added to each sample to quench the reaction and the entire mixture was centrifuged at 16,000 x g for 20 minutes at 4 °C. The supernantant of each sample was then removed and frozen overnight at -80 °C.

2.3. Isoelectric Focusing

Lysate was thawed and diluted with rehydration buffer containing 1.1X ZOOM® 2D protein solubilizer 1, 2M DTT, 1.0% pH 3-10 ZOOM® Carrier Ampholytes (ZM0021), 0.01% Bromophenol blue, and DI water as outlined by the ZOOM® IPGRunner™ protocol. The final concentration of protein of each sample was 80 µg/ml. 140 µl of the rehydration buffer mixture was then added to independent wells of a ZOOM® IPGRunner™ cassette (ZM0003). Linear pH 3-10 ZOOM® strips were placed in each well allowed to rehydrate with the rehydration buffer for 1 hour. After rehydration isoloectric focusing was preformed on all wells simultaneously by passing current through the wells and stepping up voltage sequentially from 200V to 2000 V according to protocol B in the ZOOM® IPGRunner™ protocol. Strips were then removed from wells and frozen overnight at -80 °C.

2.4 Two-dimensional gel electrophoresis

ZOOM® strips were loaded into a NuPAGE® Novex 4-12% Bis-Tris ZOOM® gel and allowed to thaw for 15 minutes in an equilibration buffer containing 4X NuPAGE® LDS sample buffer and NuPAGE® sample reducing agent at concentrations described in the ZOOM®

IPGRunner™ protocol. SDS-PAGE was then preformed at 200V for 50 minutes. Gels were stained with a SilverQuest® silver staining kit.

2.5 Gel Image analysis

Pictures of 2D gels were analyzed with Delta2D image software (version 3.6; Decodon, Greifswald, Germany) and the grayscale value of each protein spot analyzed as an indication of both protein presence and prevalence (Berth et al., 2007).

3. Results

Chemical conditions in both seawater treatments were fairly stable throughout the experiment (Table 1). The control treatment had a mean pCO_2 of 853 ± 58 µatm, which is significantly higher than open ocean conditions of ~400 µatm (Doney et al., 2009), despite my effort to equilibrate the tank with ambient air (400 ppm CO_2). However, 800 µatm of CO_2 is not necessarily out of the range of what these organisms experience in the Salish Sea due to freshwater inputs and stagnation which result in pCO_2 fluctuations ranging from 350 to 1200 µatm (Carrington et al., unpublished data). The experimental treatment had a mean of 1834 ± 277 µatm which was more significantly different than the control treatment (ttest, $p < 0.001$). This treatment level is fairly extreme when compared with open ocean levels today and are consistent with IPCC predictions for the open ocean in 150-200 years (IPCC 2013).

No results yet from protein analysis.

4. Discussion

Nothing to discuss as of yet. I will be finished with protocol on Monday, 12/2.

5. Literature cited

Bell, E. & Gosline, J. Mechanical design of mussel byssus: Material yield enhances attachment strength. J. Exp. Biol. 199, 1005_1017 (1996).

Berth, M., Moser, F. M., Kolbe, M. and Bernhardt, J. (2007). The state of the art in the analysis of two-dimensional gel electrophoresis images. Appl. Microbiol. Biotechnol. 76, 1223-1243.

Doney, S. C., Fabry, V. J., Feely, R. A., & Kleypas, J. A. (2009). Ocean acidification: the other CO2 problem. *Marine Science*, *1*.

FAO Yearbook: Fishery and Aquaculture Statistics, 2009 (Statistics and Information Service of the Fisheries and Aquaculture Department, 2011).

Gazeau et al., 2007. Impact of elevated CO_2 on shellfish calcification. *Geo Res Letters*, Vol.34, L07603.

Harrington, M.J., Waite, J.H., 2007. Holdfast heroics: comparing the molecular and mechanical properties of Mytilus californianus byssal threads. Journal of Experimental Biology 210, 4307–4318.

Hwang, D.S., Zeng, H., Masic, A., Harrington, M.J., Israelachvili, J.N., Waite, J.H., 2010. Protein- and metal-dependent interactions of a prominent protein in mussel adhesive plaques. Journal of Biological Chemistry 285, 25850–25858.

Kurihara, 2008. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. *Mar Ecol Prog Ser*., Vol. 373: 275–284, 2008.

Lee, H., Scherer, N.F., Messersmith, P.B., 2006. Single-molecule mechanics of mussel adhesion. Proceedings of the National Academy of Sciences 103, 12999–13003.

Lee B. P., Messersmith P. B., Israelachvili J. N. & Waite J. H., 2011. Mussel-inspired adhesives and coatings. Annu. Rev. Mater. Res. 41, 99–132.

Lindahl et al., 2005. Improving Marine Water Quality by Mussel Farming: A Profitable Solution for Swedish Society. *AMBIO, Vol.* 34 (2), 131-138.

Meadows et al., 1998. Mussels and mussel beds (*Mytilus edulis*) as stabilizers of sedimentary environments in the intertidal zone. *Geol. Society*, *London, Special Publications*, Vol.139: 331-347.

O'Donnell, M.J., George, M.N., Carrington, E., 2013. Mussel byssus attachment weakened by ocean acidification. Nature Clim. Change 3, 587–590.

Suchanek, 1979. *The Mytilus Californianus Community: Studies on the Composition, Structure, Organization and Dynamics of a Mussel Bed*. Doctoral Thesis – University of Washington. Print.

Tomanek, L., Zuzow, M. J., Ivanina, A. V., Beniash, E. & Sokolova, I. M., 2011. Proteomic response to elevated PCO2 level in eastern oysters, Crassostrea virginica: evidence for oxidative stress. *The Journal of Experimental Biology* 214, 1836–1844.

Table 1. Seawater treatment conditions. pCO_2 was calculated with CO2sys using measured pH (NBS scale), temperature, a salinity of 30 PSU (estimated), and a total alkalinity of 2100 mmol kg^{-1} SW (estimated).

	measured pH		s.d.	measured temp, °C		s.d.	pCO2 (µatm), calculated		s.d.
control	7.86	±	0.03	16.5	±	0.1	853	±	58
treatment	7.55	±	0.07	16.5	±	0.1	1834	±	277