Bioenergetic Effects of Cutting Mussel Byssus Threads, *Mytilus trossulus*

Sam LaFramboise, Megan Dethier, Alex Lowe

Near Shore Ecology Research 2014

Spring 2014

Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250

Contact Information:

Smlfrmbs23@gmail.com

Keywords: *Mytilus trossulus*, bioenergetics, byssal threads, aquaculture

The Bioenergetic Effects of Cutting Mussel Byssus in *Mytilus trossulus*

Researcher: Sam LaFramboise

Advisers: Megan Dethier, and Laura Newcomb

Abstract

Mussels are filter feeders living and thriving in the harsh conditions of the intertidal environment. Many factors attribute to their success including aggregating in groups, hard shells, but more specifically their ability to strongly attach themselves to substrate with byssal threads. Byssus is a proteinaceous fiber excreted by the byssal foot of a mussel for attachment. It is important to understand the energy distribution *Mytilus trossulus*, by investigating the energetic cost of byssus production. In this study we manipulate *M. trossulus* into producing different amounts of byssus among three treatments over a thirty-day experiment. Three byssal removal regiments: those cut daily, those cut weekly, and those never cut, were measured and analyzed for comparisons in metric growth. The mussels with byssal threads being cut daily showed the lowest percent increases in shell length, height, width, and weight among the three treatment groups.

Introduction

The ways by which organisms expend calories plays an integral role in their survival. Respiration, consumption, movement, growth: all have an energy budget. For this reason it is important to develop an understanding of the bioenergetics of how animals like mussels allocate energy. Mussels make byssus, strong collagenous fibers used for attachment. The byssal apparatus of *Mytilus sp.* is an exceptional case of an exocrine collagen-secreting gland (Zuccarello, 1980). Byssus is composed of secretory granules of mainly proteinaceous complex, and making them may affect the animals' overall allocation of energy. Mussels assimilate food through filtration, and use energy to build shells and byssus, along with body tissue. If one of these costs increases, the others must decrease. For example, the metabolic rate of green-lipped mussels increases after feeding, but is at the maximum during byssus reattachment (Lurman et al., 2013).

In nature, the intertidal environment exposes mussels to conditions in which they may need to continually reattach new byssal threads. Mussels in the intertidal aggregate in dense beds to help minimize the impacts of high water flow (Carrington et al. 2008). Byssus production is essential because the intertidal zone is exposed to the adverse conditions of predation, tide change, wave action, and in the Pacific Northwest, considerable seasonality (Moeser and Leba, 2006). In order to filter feed in these adverse conditions, *Mytilus trossulus*, a mussel native to the Pacific Northwest, must remain well anchored (Bell and Gosline, 1995). For the species *Mytilus californianus,* the investment in many strong byssal threads has led to its great success in wave-swept habitats (Bell and Gosline, 1997). Because mussels have a higher metabolic rate when making byssus, *Mytilus sp*. in the intertidal environment must obligate substantial energy to byssal production in order to survive.

If *Mytilus trossulus* byssal threads are severed frequently, the animals presumably will expend more energy producing new byssus. In a pilot experiment in Fall

2013, mussels with byssal threads cut daily during a five-week experiment demonstrated slightly inhibited overall growth compared to mussels with byssal threads that were never cut in that time period (McCartha 2013). I hypothesize that compared to mussels whose byssus were left intact, mussels treated by the cutting of byssus will exhibit a higher metabolic rate and lower relative growth rate, indicating a shift in bioenergetics during byssal reattachment. My experiment tests this hypothesis in a season with greater food availability for comparison to the previous work in fall conditions. Because phytoplankton is more abundant in the spring season, creating higher food availability for *M. trossulus* (Masson, 2009), I expect faster overall growth, and significant differences between experimental groups forced to produce byssus at different rates.

Methods

The methods in this experiment are based on those from the fall McCartha (2013) experiment, and have been amended for a spring season experiment.

Mussel collection and preparation: I harvested approximately eighty *Mytilus trossulus* during low tide from Argyle Creek on San Juan Island (Lat. 48°31'18.13"N, Long. 123° 0'50.22"W). Sixty mussels of similar size (approx. 2.0-3.0 cm in length) were selected from the eighty harvested for the experiment. I removed remaining byssal threads from each mussel, and tagged each using numeric tags and epoxy. Initial measurements of length (anterior to posterior), height (dorsal to ventral), and width (right to left valve) were taken of each mussel. I measured and recorded buoyant weight by keeping the mussels submerged to prevent inconsistency in weight due to water within the shell.

These measures were used as the basis for comparison and analysis of growth.

Cage construction: I constructed nine cylindrical cages (22 cm long x 10 cm diameter) using 0.5 mm flexible plastic mesh for each treatment (three replicates per treatment). The front of the units were secured with a square knot. The tops and bottoms were zip tied on one end and tied with a square knot at the opening side so that they could be opened. Five 6-inch zip ties for each cage were inserted and secured crossing through the mesh units and distanced 4cm apart.

Treatments: Mussels in "Daily" treatments were monitored for byssus production daily, and byssal threads were counted and removed by cutting with suture scissors. Mussels in "Weekly" treatments were monitored for byssus production once a week and byssal threads were counted and removed. "Never" treatment replicates were monitored for byssus production and presence or absence of byssal threads was documented, but not counted and cut until the final day of the experiment.

Five mussels were randomly selected for each replicate, totaling 45 mussels. Mussels for the treatment group that would not have their byssus removed, treatment Never, were secured to the zip tie using epoxy because they could not reach any other mussel or cage sides. The other two treatment groups, Daily and Weekly, were attached to approximately one inch leashes of nylon squid fishing line. The leashes were tied to the zip ties, with those mussels attached to the other end of the fishing lines using epoxy. I hung the nine cages from the bridge located at the floating dock of Friday Harbor Laboratories (Lat. 48°32'41.87"N, Long. 123°0'44.69"W). This set up is identical to the Fall quarter experiment, save in late Spring season to observe effects of increased food availability.

Byssus production was monitored for thirty days. I cut and count the byssus of "daily" treatment each day. At the end of the first week I cut and count the byssus of both the "daily" and "weekly" treatment, as well as measure all three treatments for length and height. The byssus of the "never" treatment mussel were not cut until the final day of the experiment.

Process mussels: Fifteen of the sixty numbered mussels not used in the experiment were selected and weighed to determine the relationship of shell and biomass for our three treatments. This sample is represented as the "pre-treatment" group. The shell length, height, and width were measured and recorded. Gonads and whole tissue biomass were separated from the shell and dried at 60°C for a week to determine dry weight of the gonad and combined tissue. The left valve, right valve, and total shell weights were measured.

After the experimental thirty day period, the mussels in each treatment were cleaned of epoxy and remaining byssus and weighed for final buoyant weight to determine changes in shell mass. The length, height, and width were measured and recorded. Gonads and whole tissue biomass were separated from the shell and dried at 60°C for three days to determine dry weight of the individual mussels. Gonadosomatic index was calculated using: GSI = [[Gonad Weight (g)/ Total Tissue Weight (g)] x 100]. Condition index was calculated using: CI = [Final Weight (g)/Final L^3 (cm)].

Results

The number of byssal threads produced over the thirty-day experimental period varied significantly with treatment (1-way ANOVA F= 63, p <0.001). Animals in the Daily treatment produced more byssus than either the Weekly or Never animals (Tukey post-hoc test, Never-Daily P<0.001, Weekly-Daily P<0.001, Weekly-Never P=0.2).

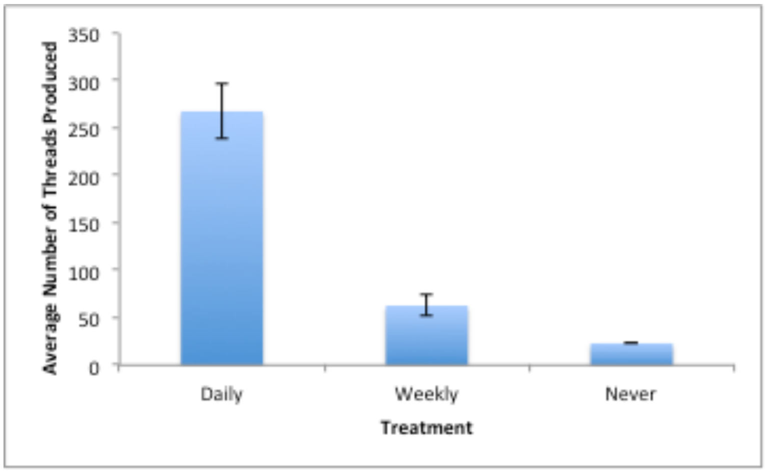

Figure 1. Average and standard error of number of threads produced by mussels in each treatment over thirty days. Significant differences occur in the Daily treatment.

After thirty days, animals in each treatment (Never, Weekly, Daily) grew different lengths, heights, and widths, although none of the metrics were significantly different (one-way ANOVAs, p values >0.05). The Never treatment showed the greatest shell length increase, Weekly slightly less, and Daily showed the lowest increase in shell length. Mussel shell height increase between treatments did not follow the same trend. The Never treatment shell height increased slightly less than Weekly, with Daily increasing the least. For each treatment, mussel shell width increases in the same order.

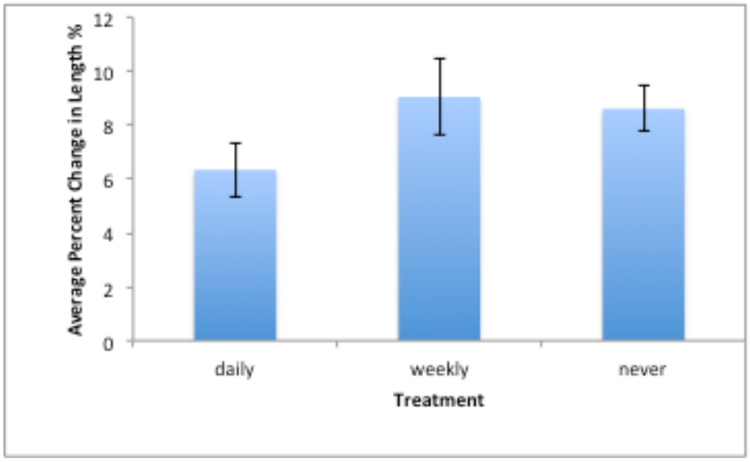

Figure 2. Average and standard error of percent change in shell length (%) over thirty days. No significance between treatments.

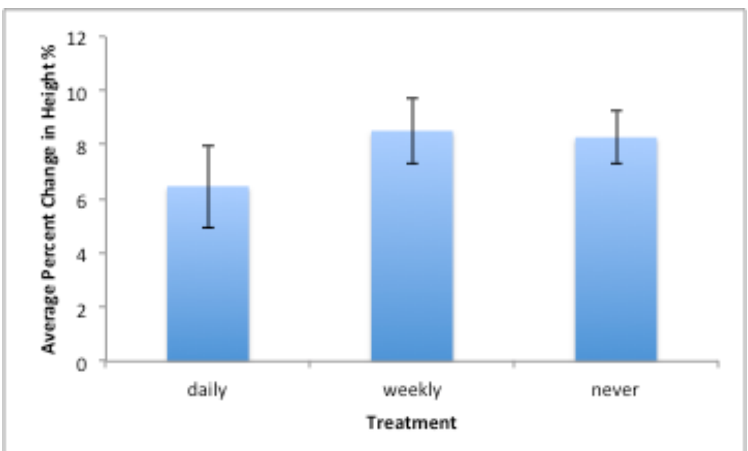

Figure 3. Average and percent error of percent change in mussel shell height of thirty-day experiment. No statistical significance preset between treatments.

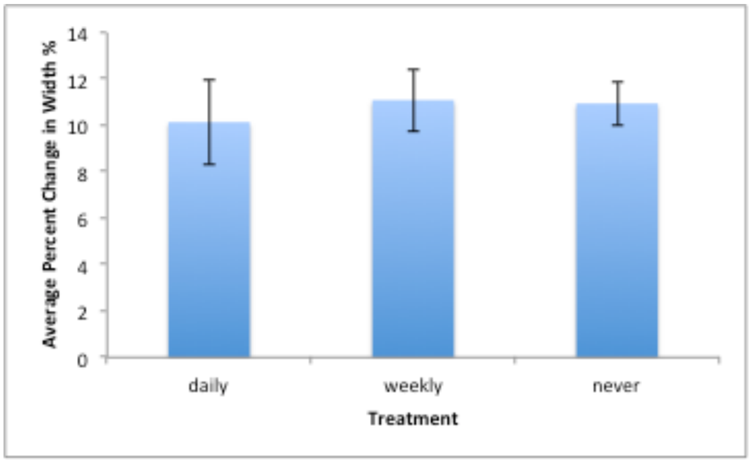

Figure 4. Average and percent error of percent change in mussel shell width over thirty days. No significance between treatments.

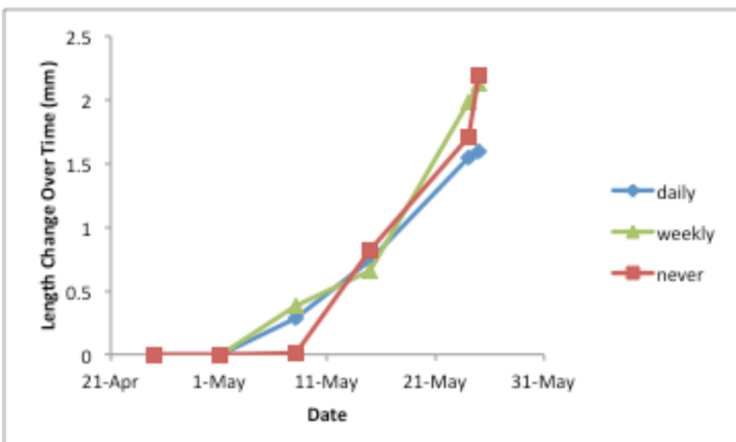

Figure 5. Trend through time in average mussel shell length (mm) between treatments over thirty days.

Average percent increases in the live buoyant weight for each treatment showed no significant difference, but did follow the same trend as all other metrics, with Daily increasing the least. The total shell mass of each mussel was divided by its final length, resulting in no statistical significance among treatments (1-way ANOVA, P = 0.44, F [change for each]= 0.85). A Gonad Index and the Condition Index were not significant and revealed no trending (1-way ANOVA, P = 0.81, f = 0.21), (1-way ANOVA, P = 0.32, f = 1.2).

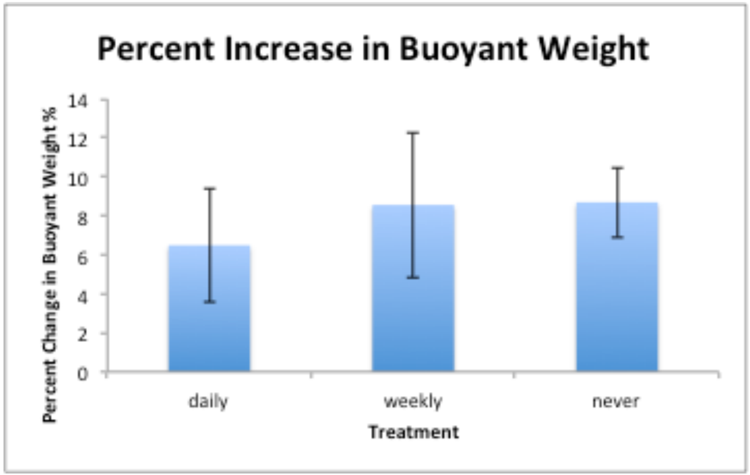

Figure 6. Average and standard error of percent change in buoyant weight of mussels in each treatment over thirty days. No significance is present.

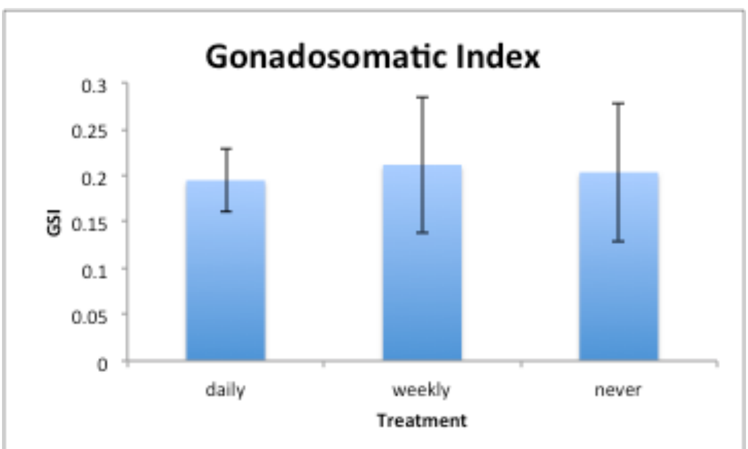

Figure 7. Average and standard error of percent of gonad in total tissue in each treatment after the thirty-day experiment. No significance is present.

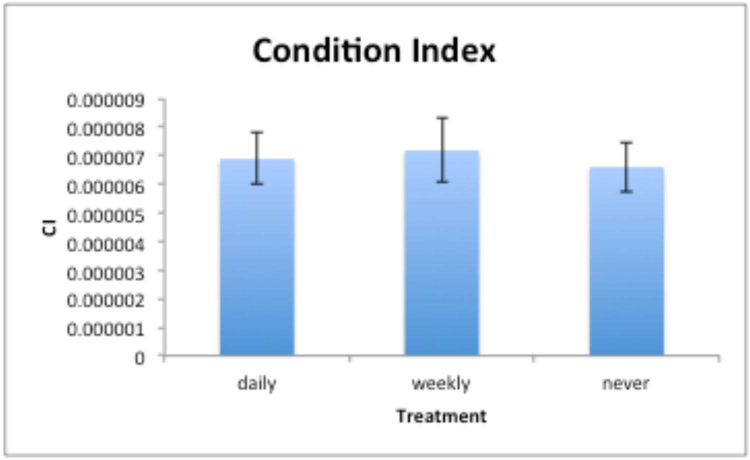

Figure 8. Index and percent error of the total tissue mass divided by the final shell length on average of each treatment after the thirty-day experiment. No significance is present.

Discussion

Because mussels produce byssus when unattached, by cutting byssal threads of *M. trossulus* every day, it is certain that they will produce more byssus than *M. trossulus* with threads left intact ($P < 0.001$). Although few of our metrics of mussel growth were significant, mussels with byssal threads being cut every day showed trends in shifting bioenergetics towards less overall growth. The trends in growth suggest that a study conducted over a longer time frame, including larger sample sizes, would likely produced the anticipated amplification of differences in growth among the treatments.

The Daily treatment was consistently lower in overall growth than the other treatments. Weekly and Never were inconsistent, but grew more than Daily. From May

17 through the end of the experiment, all data points show Daily length quantities lower, and increasing at a lesser rater than Weekly and Never (figure 5).

During the last 16 days of the experiment there was an observable phytoplankton bloom that enveloped the cages. At this time there was a spike in mussel shell growth (Figure 5). While no tests were done to confirm the type of phytoplankton in the bloom, it likely provided the mussels with an abundance of food, thus accelerating mussel growth during this time.

In McCartha (2013) fall experiment, shell length, tissue mass, shell mass, and condition index were all indicative of less growth in the Daily treatment than Weekly, and Never, as I found in the spring. Differences between the fall and spring experiments could include higher flow forces in the fall, and higher food availability in the spring, placing less emphasis on resistance and byssal production. Additionally, in the fall there was disturbance from debris colliding with and entangling the cages. Perhaps such disturbances generated a greater cost to overall mussel growth, because they had to prioritize byssal production as a means of survival.

Because the sample sizes in our experiment were small, variability among individual mussels strongly impacted treatment averages. If sample sizes were increased, significant treatment effects would be clearer. If this experiment were to be conducted over the course of a year or even a full season, differences in mussel growth in each treatment would be further spread.

Acknowledgements

I would like to thank Friday Harbor Laboratories, which granted me a scholarship to help cover the costs of my research this quarter. Also, thank you to Laura Newcomb who mentored me through this research experience. Marc Brown, and Neropam Nigam also helped me collect data during the thirty days of collection, thank you. Thank you Hilary Hayford for Excel advice.

References

Carrington, E. (2002) The ecomechanics of mussel attachment: From molecules to ecosystems. *Integrative and Comparative Biology*, **42**(4), 846-852.

Carrington, E., Moeser, G.M., Thompson, S.B., Coutts, C.L., Craig, C. A., (2008) Mussel attachment on rocky shores: the effect of flow -on byssus production. *Integrative and Comparative Biology*, **48**(6), 801-807.

FHL Weather Station: main tower. Wind Speed, Wind Chill, Air temperature, Relative humidity. 2013-2014. http://wx.fhl.washington.edu/vdv/VV_Frame.php

Masson, D., Pena, A. (2009) Chlorophyll distribution in a temperate estuary: The Strait of Georgia and Juan de Fuca Strait. *Estuarine Coastal and Shelf Science*, **82**(1), 19-28.

McCartha, M., Harrington, K. (2013) FHL Fall Quarter Marine Environmental Research: Mussel Bioenergetics, The energetic cost of mussel byssus production in *Mytilus trossulus*

McClain, C.R., Gullett, T. (2012) Increased Energy Promotes Size-Based Niche Availability In Marine Mollusks. *Evolution*, **66**(7), 2204-2215.

Moeser, G.M., E. Carrington (2006) Seasonal variation in mussel byssal thread mechanics. *Journal of Experimental Biology*, **209**(10), 1996-2003.

Moeser, G.M., H. Leba (2006) Seasonal influence of wave action on thread production in *Mytilus edulis*. *Journal of Experimental Biology*, **209**(5), 881-890.

Zuccarello, L.V. (1980) The Collagen Gland Of *Mytilus Galloprovincialis* - An Ultrastructural And Cytochemical Study On Secretory Granules. *Journal of Ultrastructure Research*, **73**(2), 135-147.