

# Variation in Byssal Thread Production by *Mytilus trossulus* with Temperature

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## Abstract

Mussels attach to substrates by means of byssal threads. The amount of threads produced influences the strength of attachment. In this experiment, we looked at the effect of temperature on the number of byssal threads formed by the marine mussel species *Mytilus trossulus*. We predicted that higher temperatures would limit the number of byssal threads produced. Mussels were placed in a flume set to a controlled temperature for each trial. Trials were performed at 10, 14, 18, 20, 22 and 25 degrees Celsius. Threads produced by each mussel were counted 11 times over a 24 hour period. Scanning electron microscope photos were also taken of threads from each temperature to compare possible visual differences. Our results suggest that total mean threads produced by *M. trossulus* peak around 18 degrees Celsius, and decrease as temperatures increase or decrease. The importance of knowledge on factors that affect byssal thread formation becomes increasingly important as global water temperature is expected to rise. Mussel attachment is important for ecological reasons, because mussels and the ecosystem that they live in depend on the formation of thick mussel beds. This information is also useful for mussel aquaculture, as the raising of farmed mussels depends on the ability of mussels to attach to substrates.

## Intro

The intertidal environment is hydro-dynamically stressful. Organisms that live in this zone have developed various ways to deal with this stress, and one of these adaptations is the byssal thread of the mussel. Mussels use byssal threads to attach themselves to hard substrates to prevent dislodgement. Byssal threads are collagenous and make up a structure known as the byssus (Lachance, 2008). The byssal thread has three regions: the ridged proximal region, smooth distal region, and the plaque, which attaches the thread to a substrate. The threads are proximally attached to a “stem” which attaches to 12 retractor muscles inside the mussel (Waite, 2012). The threads are made inside the foot of the mussel as it is extended, a process which generally takes about 3 minutes (Waite, 1992).

There has been substantial research into which factors affect the strength and number of byssal threads formed by mussels, particularly *Mytilus edulus*. This research includes influence of wave stress on attachment strength, which shows a relationship between lower attachment strength and higher wave action. (Carrington 2002a, Carrington 2008, Moeser 2006a, Carrington 2009). There is also research that shows strong seasonal effects on mussel displacement, with the highest attachment occurring in spring (Moeser 2006b).

Seasonality does seem to affect mussel attachment, and so we can now explore which factor of seasonality is most affecting mussel attachment. Previous experiments on *M. edulus* have showed varied results in temperature effects on attachment strength (Young, 1985, Carrington, 2002a). Seasonal temperature might be one factor in tenacity of *M. edulus* attachment, with attachment strength peaking before the water had reached

its highest seasonal temperatures (Carrington, 2002a). Little research has been done on factors influencing thread production of *Mytilus trossulus*, a close relative of *M. edulis* with a different range.

This experiment looks at the question: does temperature affect the number of byssal threads produced by *Mytilus trossulus*? The change in temperature due to climate change is predicted to be higher in Puget Sound than the global average (Snover et. al, 2005), and it is important that we understand the effects of these temperatures on the organisms of this region such as *Mytilus trossulus*. With our research we are hoping to provide quantitative information on byssal thread number variation that will further increase understanding of *Mytilus trossulus* and other saltwater mussels.

## Methods and Materials

*Introduction to Mytilus trossulus:* Also commonly known as the bay mussel, *Mytilus trossulus* is native to much of the North Pacific Ocean, including Puget Sound (Braby and Somero, 2005).

*Mussel collection and housing:* All mussels for the experiment were collected from Argyle Creek, a small saltwater stream running across Jackson Beach on San Juan Island, Washington. Each mussel was measured to be between 40 and 50 millimeters in length. A total of 80 mussels were collected for our experiment. The mussels were stored in ocean water under the dock of Friday Harbor Laboratories when not being tested.

*Experimental Preparations:* 12 new mussels were used for each trial, and before each trial, the height, width and length of each mussel was measured and recorded. All byssal

thread material was cut from each mussel using scissors. All limpets or barnacles stuck to the shell were either pulled off or removed with a razorblade. To set up each trial, we first attached the mussels to a mussel rack that was constructed prior to the experiment (fig. 2). The mussel rack consisted of a plastic egg crate top and sides with hanging zip ties to attach the mussels to, and a clear plexiglass bottom. The mussel rack was held together using hot glue and zip ties. The mussels were attached to the zip ties hanging from the top of the rack using cyanoacrylate glue, and the bottom of each mussel was measured to lay 6.5 millimeters off the plexiglass bottom of the mussel rack.

The mussel rack was placed in a small flume tank set to a constant flow velocity of 6 centimeters per second. The flume was filled with filtered saltwater directly from the Salish Sea system, and was kept at a controlled temperature using a Thermoscientific Neslab RTE10 Digital Plus combination heater and cooler. New seawater was constantly cycled through the flume for the duration of each trial. A thick sheet of cardboard honeycomb was placed in between the mussel rack and the water input tube to aid with water mixing. Two aquarium oxygenators were placed in the tank to provide oxygen.

*Experimental Design:* Each temperature trial was 24 hours. The number of byssal threads that had been attached to a substrate were counted and recorded for each mussel at hours 1, 2, 3, 4, 5, 6, 10, 14, 18, 22 and 24. The mussel rack was removed from the tank, and byssal threads from each trial were removed from the mussels and kept for further inspection with a scanning electron microscope. Threads and plaques from each trial were later photographed using the scanning electron microscope and compared.

*Statistical Analysis*- Total mean threads produced per trial were analyzed with a one way ANOVA. An ANCOVA (analysis of variance) was performed on the mean thread counts per hour to compare thread counts over time by trial. T tests were performed on number of mussels attached per hour to compare number of mussels attached between different treatments. The number of threads produced as a function of mussel size was analyzed with a linear regression for each size measurement (height, width, length).

## Results

The highest mean total thread production occurred at 18 degrees Celsius (fig. 5). When placed in higher and lower temperatures, the total mean thread count was lower. The total mean thread count tended to decrease as temperatures increased from 18 degrees. However, total mean thread count was measured to be higher at 14 degrees Celsius than at 10 degrees Celsius, so thread count didn't decrease as temperatures decreased from 18 degrees. Total mean thread production per trial as a function of temperature was found to be significantly different between trials (ANOVA,  $P < .0001$ ). No threads were produced at 25 degrees Celsius. Also, during the 25 degree trial, mussels were not observed to put their foot outside their shell or to open their shell, while in all other trials this was observed frequently.

Percentage of mussels attached after 24 hours as a function of temperature (Fig 8), however, was found to be significant (ANOVA,  $p < .0001$ ). Higher temperatures (20, 22, and 25) degrees had the lowest percentage of mussels attached, while 18 and 14 degrees had the highest percentage of mussels attached (92%). Slightly fewer mussels attached at 10 degrees than at 18 and 14. This suggests that the high thread production wasn't just

due to a few mussels, but that a high percentage of mussels attached in trials with high overall thread production.

We also found that it took more time for mussels to produce threads when temperatures were higher (fig. 4). Mean thread count over time by trial was found to be significantly different (ANCOVA:  $p < .0001$ ). This seems to show that, besides there being a relationship between thread count and temperature in our trial, the effect that temperature has on thread production also varies with time.

Increases in temperature had more extreme effects on temperature than decreases in temperature. When compared with 18 degrees, 22 and 20 were found to be significantly different (2 t post test,  $p < .0001$  for both), while 18 and 14 were found to be significantly different, but not as much ( $p = 0.0028$ ) and 10 was found to not be significantly different ( $p = 0.2664$ ).

One extraneous factor that we considered may have influenced the production of threads by mussels was size. The only aspect of mussel size which was significantly different per trial was width (ANOVA:  $p = 0.0004$ ) When analyzed using a nonlinear regression, number of threads produced did not have a significant correlation with mussel length, width, or height (table 1). Based on this analysis, size of mussels didn't correlate with number of threads produced.

*Scanning electron microscope results:* When analyzed with the scanning electron microscope (S.E.M), there didn't seem to be any noticeable difference between threads produced during different temperatures. Threads produced at higher temperatures did look as if the outer cuticle surface may be peeling (fig. 9), although it is likely that this

was just a result of the drying process and happened prior to the experience. There also didn't seem to be any significant difference between photos of byssal plaques taken. Although the pictures suggest that some plaques are distorted (Fig. 10), this is likely just a result of the drying process.

## Discussion

Based on our trials, an overall positive correlation was found between total thread production and temperature. The strongest total thread production response was measured at 18 degrees, while fewer threads were observed at temperatures which were higher or lower than that. Generally, thread number decreased as temperatures increased or decreased from 18 degrees, with a more extreme change in thread count observed as temperatures increased. No threads were produced at 25 degrees. We also found that threads were generally produced more slowly in higher temperatures, showing that time might also play a factor in how mussels are affected by temperature.

Based on previous research, the response of our mussels to changes in temperature seems likely. Pearce (1969) found byssal thread attachment in *M. edulis* to be unusual over 24 degrees. It is possible that this lack of byssal thread production after a certain temperature threshold is due to decreased rates in metabolism. Temperature is thought to be the top factor influencing metabolic rate in mussel species (Jorgensen 1966). The upper range at which *M. edulis* is estimated to function is at temperatures from 27 degrees Celsius (Read and Cumming 1967) to 28.2 degrees Celsius (Wallis 1975). It is possible that *M. Trossulus* has too low of a metabolic rate at 25 degrees Celsius to produce any threads, and so no threads were produced at this temperature.

Another interesting finding is that temperature effects on thread production vary over time. Mean thread counts seem to become more varied as time goes on, suggesting that when *M. trossulus* is placed in higher temperatures, it takes a longer time to attach and produces threads more slowly than it would at lower temperatures. This suggests that the adverse effects of warmer temperatures on mussels may increase with time spent in that temperature.

It should also be noted that one mussel was found spawning during our 14 degree trial, and two were found spawning during the 18 degree trial. There is some evidence that *M. edulis* thread production and attachment strength increases around periods of high reproductive condition, and that spawning periods occur during times of year when sea surface temperature is high (Carrington, 2002a). The reasons for this are not fully understood and need to be more fully researched. However, the high reproductive condition of mussels in warm water could have contributed to a difference in thread production during these trials than would have occurred if none of the mussels are spawning.

Besides number of threads produced, strength of threads produced is also an important factor of mussel attachment. Although thread strength wasn't directly measured in this experiment, threads from different temperature trials were viewed under a Scanning Electron Microscope and compared. No noticeable difference in threads was observed between trials. However, threads produced by spawning mussels during the 14 degrees Celsius trial were thinner than those produced by non-spawning mussels during the same trial (fig. 9). This is interesting because other sources have suggested that spawning may lead to higher attachment strength, in terms of number of threads and

strength required to pull an attached mussel off of a substrate (Carrington, 2002a). However, other sources suggest that the strength of individual threads is weaker during spawning (Lachance, 2008). It is possible that more threads are produced during times of high reproductive condition, but that these threads are weaker.

If temperature does influence the production of byssal threads in mussels, this could have implications for the ecology of intertidal systems. Mussels such as *M. trossulus* are a foundation species of the mid-intertidal zone due to their abundance, as well as their formation of thick beds which are used by other organisms for shelter (Denny and Gaylord 2010). Also, there is some evidence that the formation of mussel beds allows mussels to control the impact of the force of flowing water, preventing dislodgement (Carrington 2002b). The intertidal zone already experiences a wide range of temperatures, (Lennon 1995). However, the expected worldwide water temperature rise may have an adverse effect on mussel attachment, limiting their ability to form mussel beds, which would alter the structure of the intertidal community as a whole.

Changes in mussel attachment due to temperature would also have effects on mussel aquaculture. Although *M. trossulus* isn't as common in aquaculture as other species, such as *M. edulis* or *M. californianus*, it is used in some mussel farms (Mueller, 2007). Although different mussel species have different temperature tolerance, the possibility that temperature may affect mussel attachment is important for all species of cultured mussels, because the farming of mussels depends on the ability of mussels to attach to a substrate.

The mussel thread production observed in this experiment showed a correlation between temperature and thread production, with mean thread productions peaking at 18

degrees Celsius. Fewer threads were produced at higher and lower temperatures, suggesting that temperatures that are too high or too low may inhibit thread production. Hopefully more research can be done on the effects of byssal thread production by *M. trossulus* which can further refine the boundaries of the relationship between temperatures and thread attachment, as well as attachment strength.

### Acknowledgements

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### Figures



Fig 1: A diagram showing placement and structure of the thread plaque which *Mytilus trossulus* uses to attach to substrates.

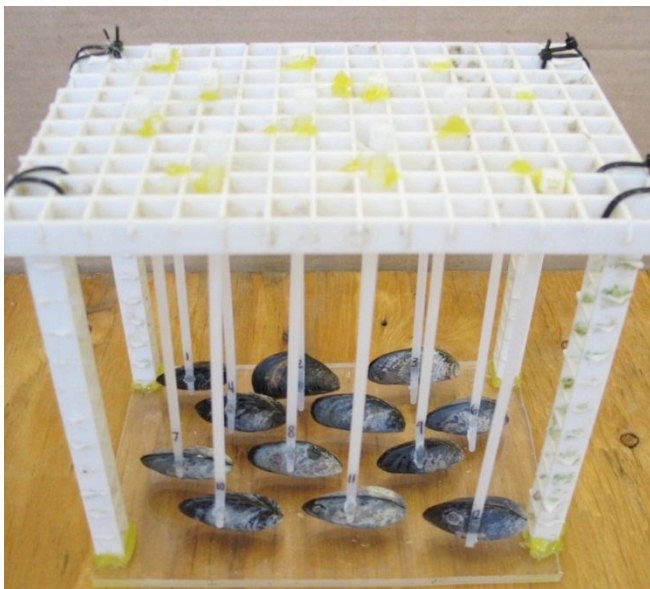


Fig. 2: mussel rack used in experiment. Mussels were glued to numbered zip ties hanging from top of mussel rack for the entirety of each 24-hour trial.

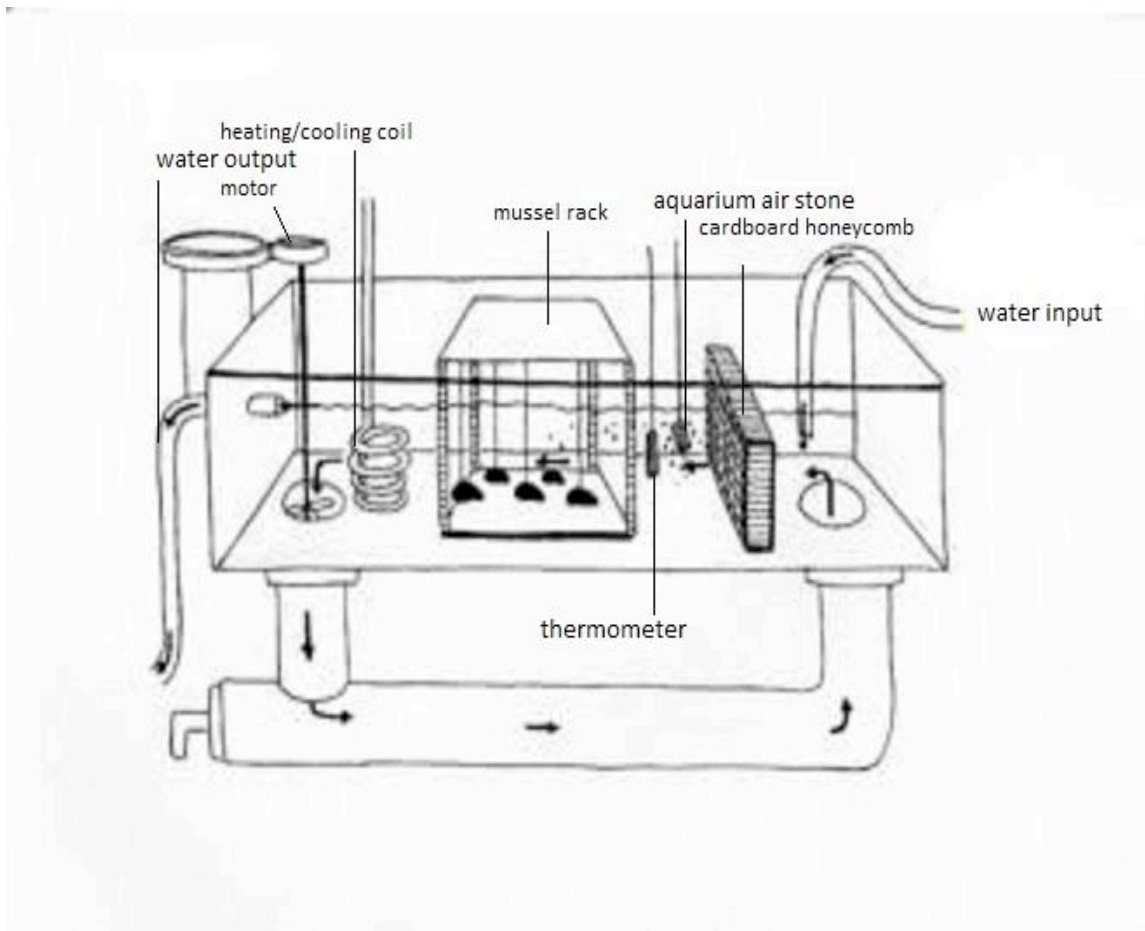


Fig. 3: Experimental setup. Arrows indicate direction of water flow.

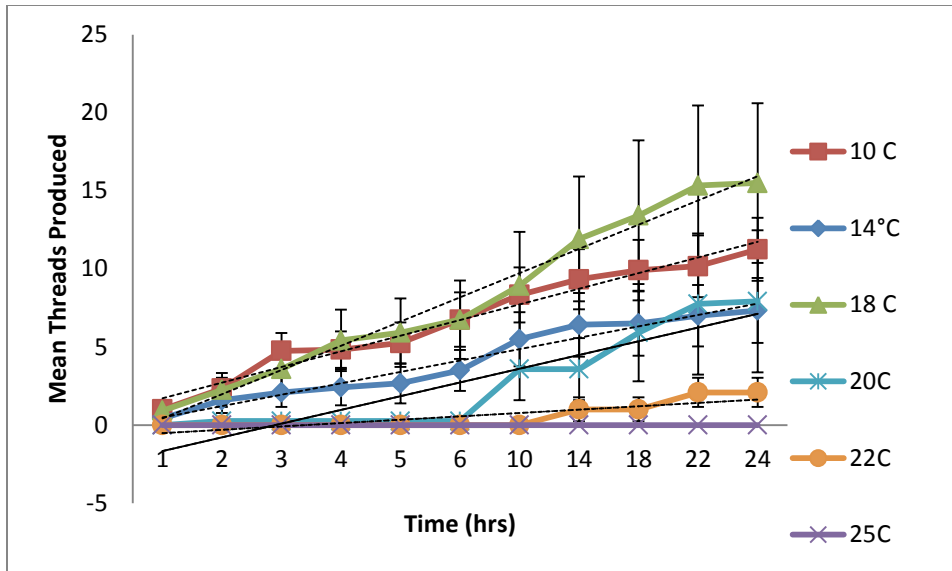


Fig. 4: Mean number of threads produced per hour by all mussels. Symbols represent mean byssal threads made by all 12 mussels at each observation  $\pm$  standard error. Lines of best fit have been added to show trends. Thread production was lowest at the two highest temperatures tested, and was highest at 18 degrees. Note that no threads were produced at 25 degrees Celsius. The data shows significance between mean threads per temperature and time, with  $F(4, 50) = 10.5143$  and  $p < .0001$ .

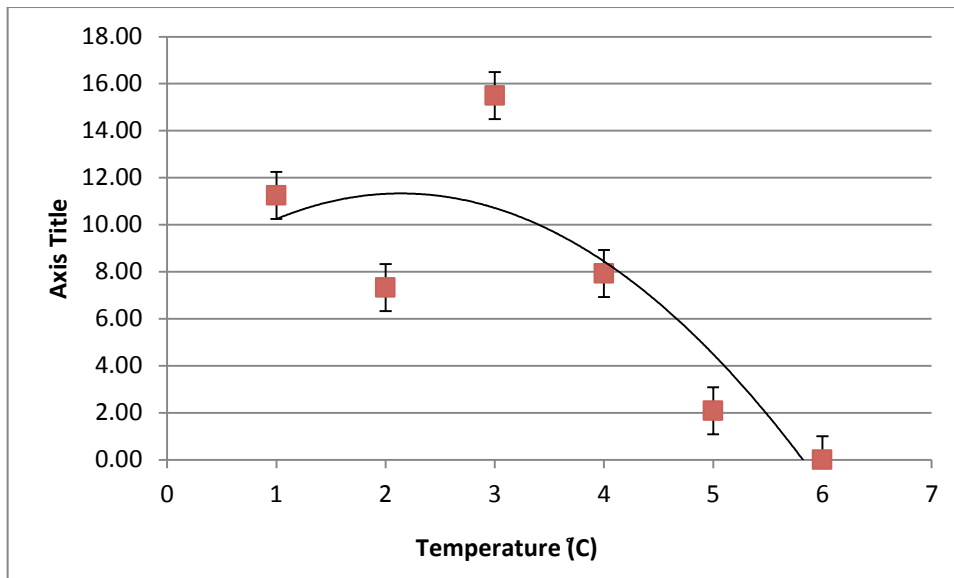


Fig. 5: Total mean threads produced by all mussels after each trial  $\pm$  standard error. Significance was found between temperature and total thread production after ANOVA analysis, with  $p < 0.0001$  and  $F(5, 60) = 12.81$ .

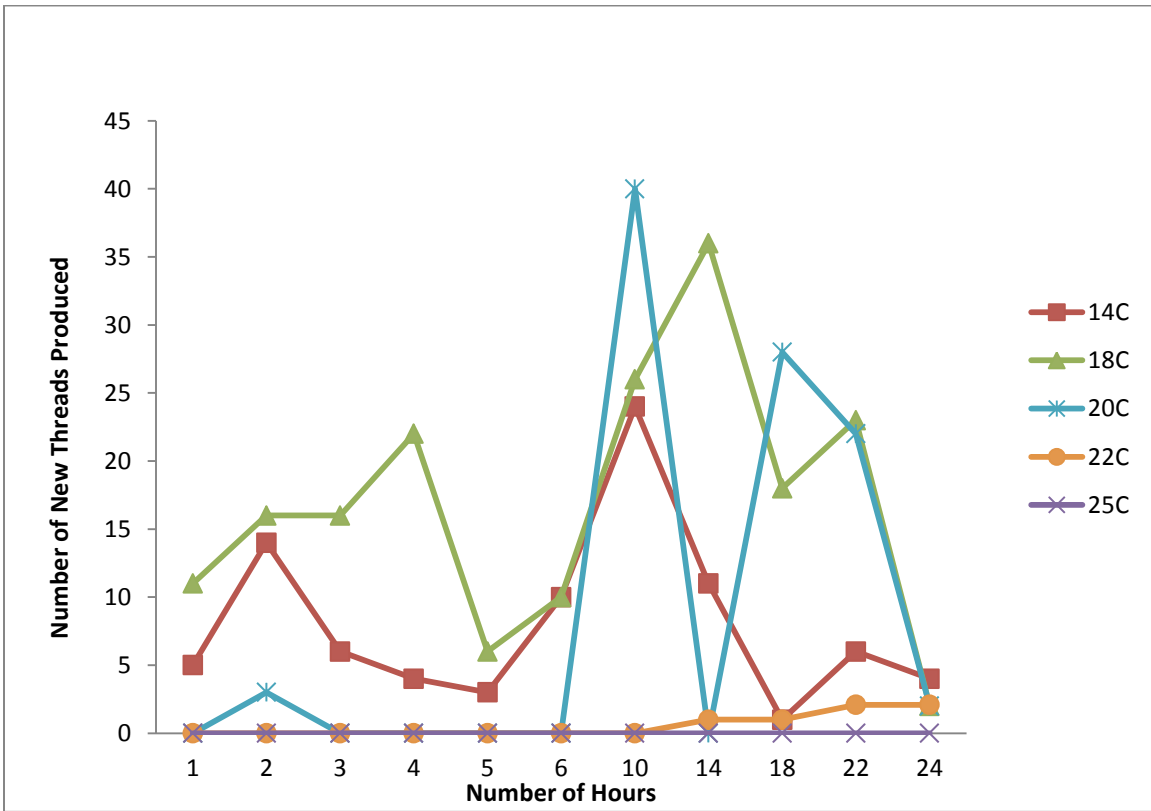


Fig. 6: New threads produced per trial over a 24-hour period. Symbols represent all new threads produced by all 12 mussels at each data point. Note that no threads were produced at 25 degrees Celsius.

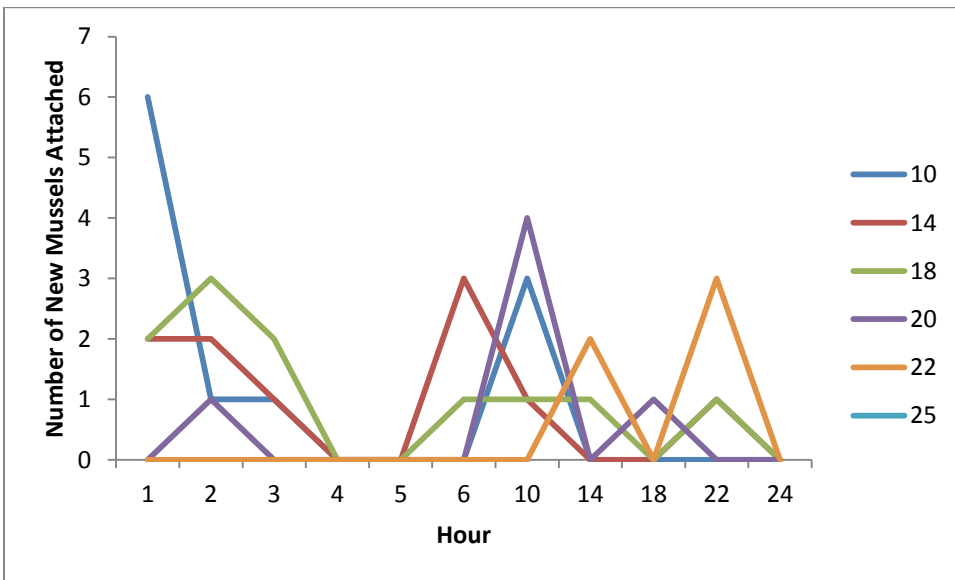


Fig. 7: Number of new mussels attached at each hour.

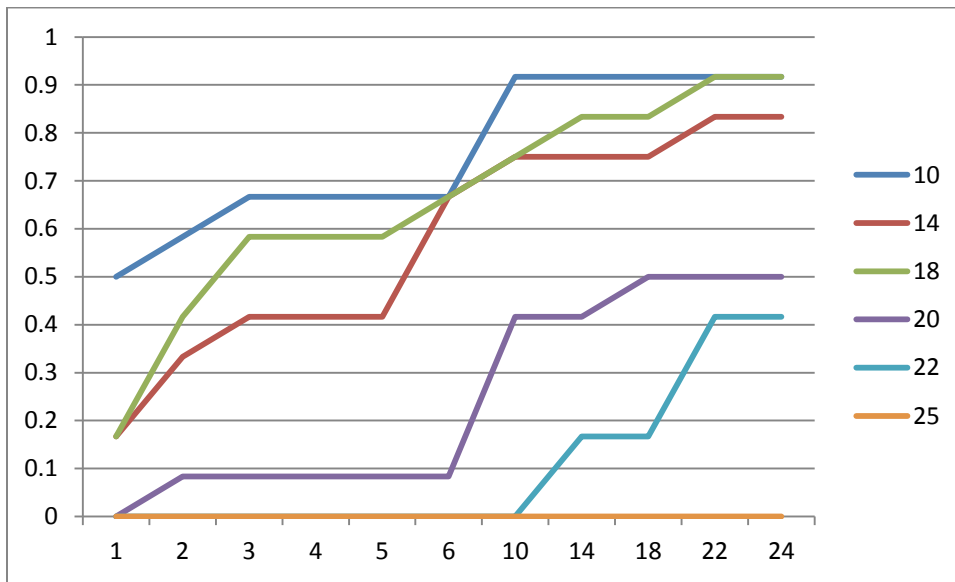


Fig. 8: Total percentage of mussels attached at each hour for four different temperatures. Note that no mussels attached at 25 degrees Celsius.

Table 1: Average mussel sizes per trial

Trial	Average length (mm)	Average width (mm)	Average height (mm)
10° C	44.58±2.75	16.92±1.62	21.33±1.56
14° C	42.08±2.47	14.83±0.83	19.5±2.65
18° C	42.17±3.76	14.25±3.55	19.42±3.82
20° C	44.5±2.47	17.5±2.23	21.83±2.52
22° C	41.5±3.29	16.08±1.5	20.25±1.29
25° C	44.25±3.28	17.08±0.79	20.83±1.34
P value	0.092	0.13	0.13
R <sup>2</sup>	0.27	0.22	0.24

Table of mean mussel length, width, and height per trial ± standard deviation, with P values and r<sup>2</sup> values obtained from linear regression analysis of threads produced as a

function of mussel size. Values show little correlation between number of threads produced and size of mussel.

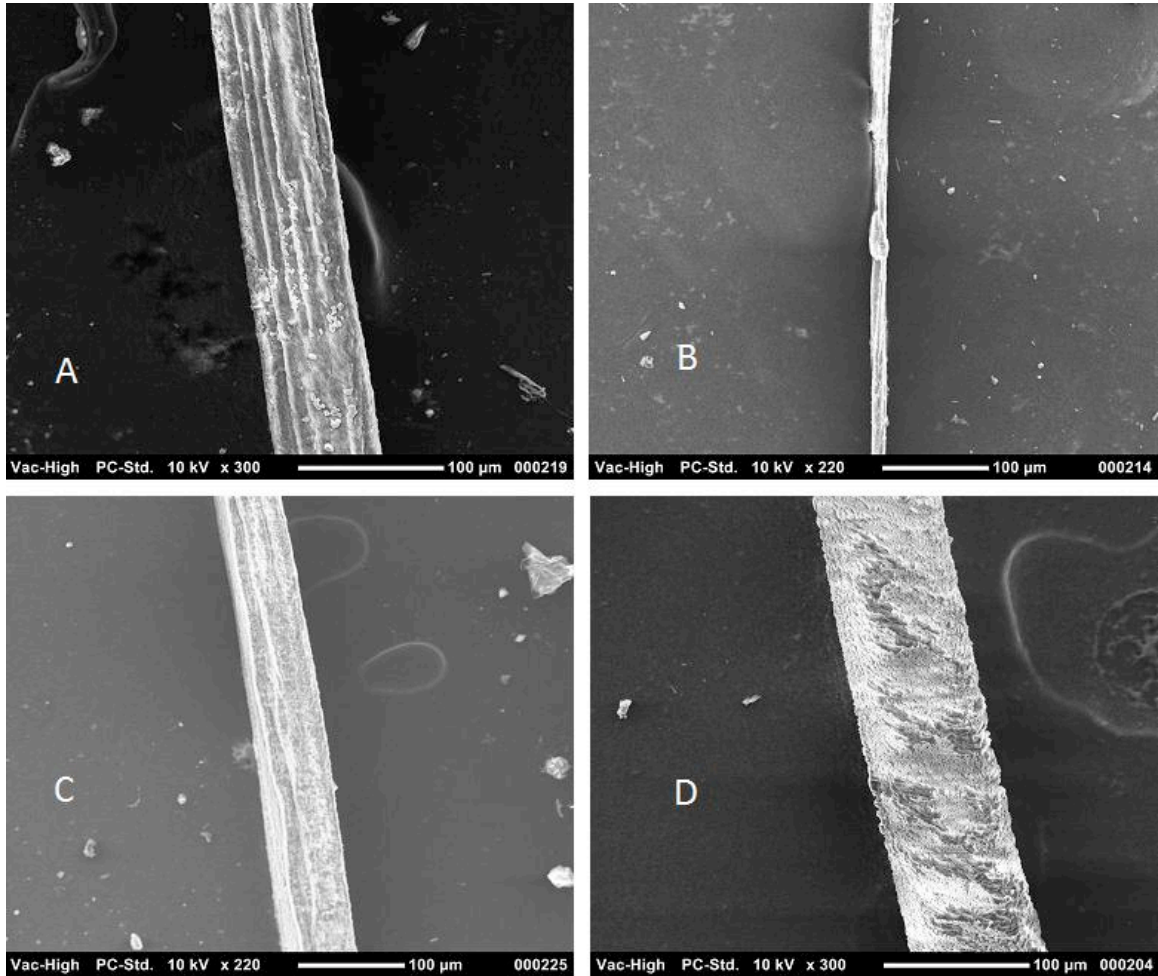


Fig. 9: S.E.M. photos of threads produced during experiment. Photo scale is indicated by labeled white bars at bottom of picture. A) thread produced by non-spawning mussel at 14 degrees B) thread produced by spawning mussel at 14 degrees C) thread produced at 18 degrees Celsius D) thread produced at 22 degrees Celsius

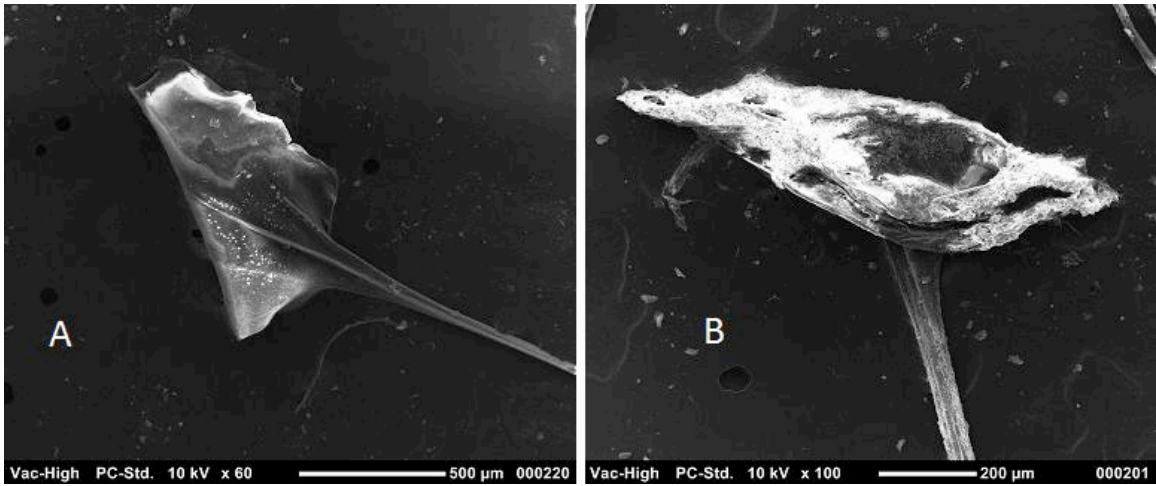


Fig. 10: S.E.M. photos of mussel byssal thread plaques. Photo scale is indicated by labeled white bars at bottom of picture. A) Plaque produced at 14 degrees Celsius B) Plaque produced at 22 degrees Celsius