The energetic cost of byssus production in *Mytilus trossulus*

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Abstract

Many stress factors affect energy budgets of species in the intertidal zone Specifically, mussels must distribute energy between processes such as attachment, shell growth, metabolism, and reproduction, all of which may be influenced not only by seasonality, but also by anthropogenic stressors. Understanding patterns of energy distribution between biologic processes is critical when deriving energy budgets of intertidal organisms. In the present study, we investigated the energetic cost of byssus production for the intertidal mussel *Mytilus trossulus*. Methods were established and administered for a four-week period, which involved exposing collected mussels to one of three, triplicate, treatments respectively. Treatments included: "Never" removing byssus, "Weekly" byssus removal, and "Daily" byssus removal. Using statistical analyses, changes in physical measurements (ie. length, width, and height), gonadosomatic index, and condition index were assessed. We identified a low amount of byssus production for mussels in the "Never" treatment, which corresponded to a lesser percent change in length (shell growth) in comparison to the other treatments. Therefore mussels may shift their energetics from producing longer shells and tissue growth

toward applying a greater amount of energy in byssus production. Combined with modeling predictions, this information will be useful for aquaculture practices as well as understanding physical changes mussels undergo in response to stressors that impact byssus production.

Key Words: Mytilus trossulus, bioenergetics, byssal threads

Introduction

As ecosystem engineers in the intertidal zone, mussels are subject to high levels of hydrodynamic disturbance, and attach themselves using collagenous threads that are molded by the foot of the mussel and extend ventrally from the root, housed inside the mussel, to the substrate (Brown 1952). Byssal threads are described by Brown (1952) as having three distinct regions which include: the distal region, which is the base of the thread closest to the mussel, the proximal region which is thinner than the distal region, and the adhesive plaque, the source of the attachment to the substrate. Because mussels rely on these threads for survival, many studies have investigated the biomechanics of byssus production and attachment.

Attachment strength varies depending on the season of growth (Price 1980, Carrington 2002). Altering attachment strength by increasing either the number of threads they produce, thread thickness, thread strength, as well as producing byssus in different seasons, may require mussels to allocate energy differently (Bell and Gosline 1997). Based on previous findings, byssus production should be considered as a factor in the bioenergetics of mussels as well as a major contributor to a mussel's survival in the intertidal zone and in aquaculture (Lurman et al. 2013, Grant 1996, Moeser and Carrington 2006, Fly and Hilbish 2013). As mussels become older, larger and more reproductively active, they have the ability to change their energy distributions from growth and byssal production, to reproduction (Fly and Hilbish 2013). However, there is a deficit in basic information regarding the bioenergetics of total byssus production, let alone the energy required for the different ways of increasing attachment strength. Overlooking changes in energy budgets may have detrimental environmental and economical repercussions. For instance, if energy requirements shift towards byssus production for survival in increasingly stressful environments, mussel reproduction could decrease (Carrington 2002), affecting the intertidal community and shellfish farm productivity.

Bell and Gosline (1995) observed that mussels not only secure their threads in the direction of the water currents, but also have the ability to vary the number of threads they produce. A larger surface area of attachment means a lesser load per each thread. Also, Moeser and Carrington (2006) found that the quality of the threads, not quantity of threads, explained seasonal variations in mussel attachment. However, neither study considered the energy required to produce byssus as the limiting factor in attachment strength, quality, or quantity. Correlating the quantity or quality of the byssal threads to the energy required to produce the threads would assist in further understanding the bioenergetics of mussels that is currently overlooked in other studies. The possible correlations may elicit patterns in energy distributions either seasonally or environmentally that could be used for assisting in seasonal aquaculture seeding and harvest times or in forecasting models of the health of mussel communities.

Past studies have determined that byssal thread structure and attachment varies between mussel species (Bell and Gosline 1997), and byssus production has a measurable energetic cost (Lurman et al. 2013). Lurman et al. (2013) examined the aerobic scope of byssal attachment and metabolic rates of the green-lipped mussel. In that study, the researchers recognized that there is a measurable energetic cost associated with byssus production, and that energies that are distributed in an organism may change, especially under different feeding regimes. Furthermore, there may be different rates of byssus production, which could change energy rates between biological processes, so it would benefit researchers studying bioenergetics of mussels to be able to measure energy costs over specific manipulated byssus production rates.

With this study, we hope to fill the information gap which links byssus production as a major factor in mussel bioenergetics. Our objective is to investigate the energetic costs that mussels incur during byssus production. Using three treatments, one for no/limited byssus

production, one for intermediate byssus production, and one for maximum byssus production, we will compare total byssus production, shell length, shell height, shell width, gonadosomatic index (GSI), and condition index (CI) between treatments, and tissue growth. If byssus production does not affect mussel bioenergetics, there should be no significant difference in comparisons signaling that the energy was allotted evenly between treatments. Combined with modeling predictions, this information will be useful for aquaculture practices as well as understanding physical limitations of mussel attachment in varying environments.

Methods

Mussel collection and preparation

Approximately 80 individual mussels, *Mytilus trossulus* native to the Pacific Northwest , were collected during low tide from Argyle Creek on San Juan Island (Lat. 48°31'18.13"N, Long. 123° 0'50.22"W) (Image 1). Mussels were cleaned and remaining roots and byssal threads were removed from organisms. Forty-five mussels were collected from a similar size class (approx. 2.0-2.5 cm in length). The mussels were labeled using numeric tags and non-toxic superglue. Initial measurements of each mussel in terms of length (anterior to posterior), height (dorsal to ventral), and width (right to left valve) were recorded. Buoyant weight was also measured and recorded. Untreated organisms were placed in a mesh bag and stored in the dock box at the University of Washington's Friday Harbor Laboratories where they could be kept alive in an environment similar to the treatment groups. We replenished mussels subject to mortality using mussels kept in the dock box as needed during the experiment in an attempt to maintain constant sample sizes.

Cage construction

We constructed nine cylindrical housing units (22 cm x 10 cm) 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 created so that they could be easily removed. Five 6-inch zip ties for each cage were inserted and secured crossing through the mesh units and distanced 4cm apart.

Treatments

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 to ensure no, or limited, byssus production because they could not reach any other mussel or cage sides. For the other two treatment groups, maximum byssus production, treatment "Daily", and intermediate byssus production, treatment "Weekly", fishing line was attached to the zip ties. Mussels were attached to the fishing lines using epoxy. Units were deployed from the bridge located at the floating dock of Friday Harbor Laboratories (Lat. 48°32'41.87"N, Long. 123°0'44.69"W) which has similar to their natural habitat (ie. temperature and water flow) as well as a constant food source for the duration of the experiment (Image 2).

Monitoring byssus production took place for five weeks. "Never" treatment replicates were monitored for byssus production and presence or absence of byssal threads was documented. Mussels in "Daily" treatments were monitored for byssus production daily and byssal threads were counted and removed by clipping. Mussels in "Weekly" treatments were monitored for byssus production once a week and byssal threads were counted and removed. Mussels in each treatment were measured for changes in length and height. Width could not be measured due to additional mass from epoxy fixation.

Mussel processing

Fifteen additional mussels not used in the experiment were selected and weighed to determine the relationship of shell and biomass for our samples. 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 four days to determine dry weight of the individual mussels.

After the experimental four-week period, the mussels in each treatment were removed 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 four 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)].

Data analysis

We used the equation [(F – I) / I) x 100] to calculate the percent change between length, width, height, and tissue mass from the first to the final week of the experiment. Grouping mussels from all treatments and reference samples, a linear regression was determined between total tissue weight and cubic shell length. Using this regression, the initial tissue mass was calculated to analyze changes in tissue mass between treatments.

One-way Analysis of Variance performed in Sigma-plot was used to determine any significant variations in length, width, height, byssus production, GSI, CI, and tissue mass between treatments. If any significance differences were present post-hoc Tukey or Holm-Sidak tests were performed to further investigate the differences between treatments. Statistical significance was set at $\alpha=0.05$.

Results

The averaged sum of threads produced for the duration of the experiment differed between each treatment (Fig. 1). A higher degree of byssus production was observed in the "Daily" treatment in comparison to the "Never" treatment (Holm-Sidak Method, $p<0.001$). Likewise, a greater number of byssus was produced in the "Daily" treatment in comparison to the "Never" treatment (Holm-Sidak Method, $p<0.001$). The averaged sum of threads was also greater for the "Weekly" treatment when compared to the "Never" treatment (Holm-Sidak Method, $p=0.016$).

The average percent change in length differed between the three treatments (ANOVA, $p<0.050$) (Fig. 2). Most notably, the percent change in length was greater in the "Never" treatment in comparison to the "Daily" treatment (Tukey Test, $p<0.050$).

Although the percent change in width was on average higher in the "Never" treatment, moderate in the "Weekly" treatment and respectively lowest in the "Daily" treatment, these differences resulted in no significance (One-way ANOVA, $p=0.072$) (Fig. 3). A similar pattern as described with percent change in width could be seen in percent change in height, also resulting in no significance (One-way ANOVA, $p=0.093$) (Fig. 4). Shell mass in the "Never" treatment was greater than the other treatments (Holm-Sidak Method, $p=0.025$) (Fig. 5).

There was little change in the calculated GSI between the three treatments (One-way ANOVA, $p=0.772$) (Fig. 6). The CI between the three treatments was higher in the "Daily" treatment, moderate in the "Weekly" treatment and respectively lowest in the "Never" treatment, although not significant (One-way ANOVA, $p=0.690$) (Fig. 7).

Using the calculated initial tissue weights, the percent change in tissue mass was greater in the "Never" treatment than the other treatments with the "Daily" treatments having the lowest

percent growth of all Shell mass in the "Never" treatment was greater than the other treatments (Holm-Sidak Method, p=0.007) (Fig. 8).

Discussion

Byssal thread production

Mussels expend variable amounts of energy during byssus production, which may change during seasons as well as with the reproductive maturity of the animal (Price 1980, Moeser and Carrington 2006). The initial objective of study was to develop a method of manipulating byssus production, assisting future studies and modeling simulations designed to examine energetic costs during byssus production. By developing this method, we will be able to determine the energetic cost associated with byssus production in relation to energy required for other processes such as growth and development of the focal species. Averaged final byssus production reflects the manipulations experienced by our samples, indicating that we now have the ability to control the amounts of byssus produced through controlling how often the mussel had to replenish byssal threads (ie. daily, weekly, never), although it was not possible to cause the mussels to produce no byssal threads at all. Using these techniques, it will now be possible to measure metabolic rate during periods of know byssus production (high or low) and thus measure the cost directly, as well as to find out exactly how that cost affects growth. It was clear from these experiments that both growth of shell, and of tissue, was reduced significantly when mussels were forced to produce high numbers of byssal threads, so the energetic cost should be substantial.

Changes in energy impacting shell growth

Changes in the distribution of energy used by an organism occur because of environmental stress, changes in natural physiological processes, or seasonal fluctuations in the physical environment (Carrington 2002). Changes in organisms' physical parameters such as tissue weight and shell production may be used as a proxy for understanding the allocation of energy although it is also possible to measure energy expenditures directly in some cases. The results of this study showed similar patterns for changes in length, width, height, and weight between treatments. For all such measures, forcing the mussel to produce more byssus resulted in a decrease in growth rate; this was significant for length and weight. Increases in shell mass reflect those seen with shell length. Meaning as mussels produced less byssus they grew longer shells. With the increased shell length, the shells inadvertently had a larger mass if the mussels grew less byssus. Further examination of changes in shell production, such as shell thickness, as a result of changes in byssus production is needed to determine if the integrity of the shell changes when energy is allotted to other physiological processes.

Moeser and Carrington (2006) suggested that there is an energetic trade off in attachment strength (byssus number and strength) and gamete production during the fall season. In the treatment groups where byssus was removed daily and weekly, dry gonad weight was affected; while the "Never" treatment averaged a greater gonad weight and the "Daily" treatment averaged the lowest gonad weight between treatments, the three gonad weights were still similar to each other and thus not statistically different. This indicates the importance of developing gonads even when faced with creating a greater byssal load. The trade-off of maintaining gonad development and byssus production first arises with shell growth as seen with the changes in shell length between treatments. Shell height and shell width had similar patterns but of a lesser caliber than shell length or total shell weight. This also indicated that mussels may allocate energy not only

between tissue and shell growth, but also distribute energy differently in the processes of creating shell structure. For this study, we concluded that activities for making longer shells were first to be forfeited as mussels were manipulated into manufacturing greater amounts of byssus during a season where developing reproductive structures were of highest priority.

Changes in energy impacting tissue development

Previous studies have identified seasonal variations affecting attachment strength and the properties of byssal threads (Moeser and Carrington 2006) and thus the energy distribution also has seasonal patterns. Understanding seasonal energetic fluctuations is important when performing ecological studies or evaluating optimum aquaculture practices. For instance, it may be better to forgo harvesting efforts during a season where the anticipated biomass of a stock is low. These times may include during the spring when higher amounts of energy are placed in byssus production and less energy is placed in tissue and gonad development (Price 1980, Moeser and Carrington 2006). The present study saw no relationship between final gonad development. However, by increasing the sample size of the reference group (initial), a linear regression analysis may be performed and applied to determine mass to length relationships of the mussels of interest. This analysis would allow future researchers the ability to better evaluate the impacts byssus production has on gonad development. The relationship for CI between our treatments was less clear. Here, it was expected that if the shell length grew, then the tissue mass would also grow to a similar affect. However, little is known regarding the rates of tissue and shell growth, which may be necessary to know. For instance, mussels may exert a lot of energy in tissue growth in comparison to shells, or the shells may be longer, but thinner or wider. These examples may complicate the relationship of shell length and tissue mass, which may not be reflected by using condition indices.

Here, we were able to group all mussels together in an attempt to determine relationships between length and total tissue mass by the end of the experiment. Although it is more desirable to use a large and randomized size sample, we were able to apply the linear regression to calculate initial tissue mass of the mussels in our treatments. By doing so, we were able to determine significant changes between treatments. Specifically we were able observed that in situations of little to no byssus production, tissue mass increases. Inversely, if mussels were prompted to increase their byssus production, tissue mass decreased.

Future studies and Applications

Studies on the energetic differences between congener mussel species determined that *M. trossulus* had lower growth of tissue and shell during summer months than did two other *Mytilus* species (Fly and Hilbish 2013). Our study used *M. trossulus* collected from their natural environment in the San Juan archipelago during fall. Using naturally slow growing species in combination with a less productive season (fall) probably reduced our ability to measure shell growth and gonad development over the four-week experimental period. Future studies using *M. trossulus* should consider a longer study period, which may result in a more consistent pattern for tissue growth and gonad development. A longer study period also allows mussels to better acclimate to their new environments, mitigating complications impacting growth, such as changes in feeding behavior and flow rates *in situ*. However, because the life span in situ of a byssal thread is estimated between four and six weeks, thread decay and natural maintenance should also be considered in future studies.

We were able to provide the "never" treatments a false sense of security in their attachment, allowing them to think that their probability of dislodgement was greatly reduced by being fixed to each zip tie respectively. However, we discovered that a number of the mussels still chose to apply their byssus to themselves if they could not reach around to the zip tie itself. Thus, the "never" treatment, although still creating a lesser amount of byssus than the other treatments, was still able to produce byssus, thereby altering the anticipated expenditure of energy for that group. Future experiments should attempt to secure mussels in a fashion that their foot will not be able to reach the zip tie at all as well as attempt to develop a way of preventing the mussel from attaching byssus to their shells. Doing so will allow future analyses of the energetic costs of byssus production to be more accurate and applicable to simulation models using these data.

This study is the first to experimentally address the energetic costs of byssus production, and how energy allocation changes between high and low byssus production rates, and the resultant effect on growth. Lurman et al. (2013) measured increased metabolic cost during periods when mussels were producing few or many byssus, but the actual production rates are unknown. Understanding and deriving energy budgets using these methods may assist in future efforts to increase mussel biomass and abundance both in nature and in aquaculture especially when combined with simulation models incorporating energetics and life history parameters. Scientific and aquaculture communities both have found simulation models useful when forecasting future survival rates and total biomass in harvestable mussel populations (Fly and Hilbish 2013, Grant and Bacher 1998). For instance, models have been affirmed to successfully predict tissue mass and growth rates in mussels based on food consumption rates in the absence of extreme turbidity (Grant and Bacher 1998). Although the use of models in both the scientific and shellfish farming fields has been increasing, few studies have included factors such as byssus production as energetic constraints, which may also influence future predictions generated by such modeling efforts.

Conclusion

We were successfully able to manipulate byssus production. Through these manipulations, we observed the prioritization of energy in the intertidal mussel *Mytilus trossulus*. As shown with this study, outside factors impacting biologic processes may alter energy allotments in marine organisms. Understanding the distribution of energy in these organisms is the first step in understanding how organisms respond to changing environmental conditions.

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Images & Figures

Image 1: Image captured from GoogleEarth of Argyle Creek on San Juan Island, located in northern Puget Sound, Washington (Lat. 48°31'18.13"N, Long. 123° 0'50.22"W). Mussels were collected from Argye Creek during low tide (11:00PM) on October 10, 2013.

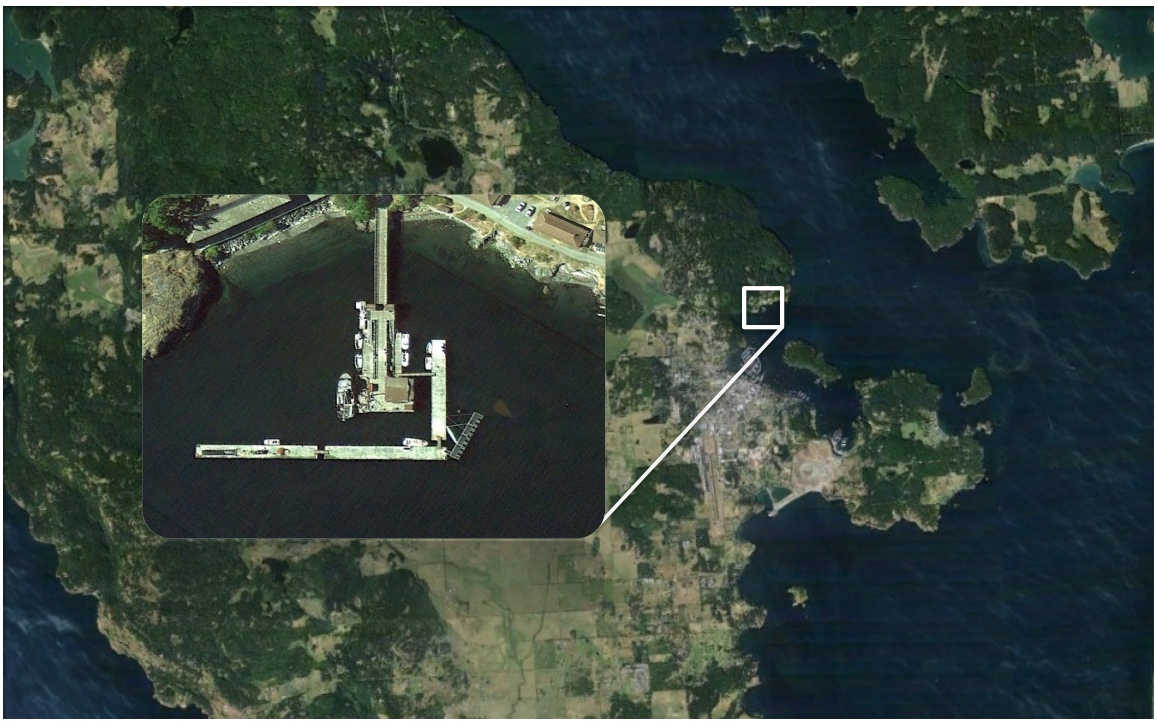

Image 2: Image captured from GoogleEarth of the floating dock of the University of Washington's Friday Harbor Laboratories, Friday Harbor, Washington (Lat. 48°32'41.87"N, Long. 123°0'44.69"W). Mussels were deployed inside their housing units from the bridge connecting the two outermost floating docks.

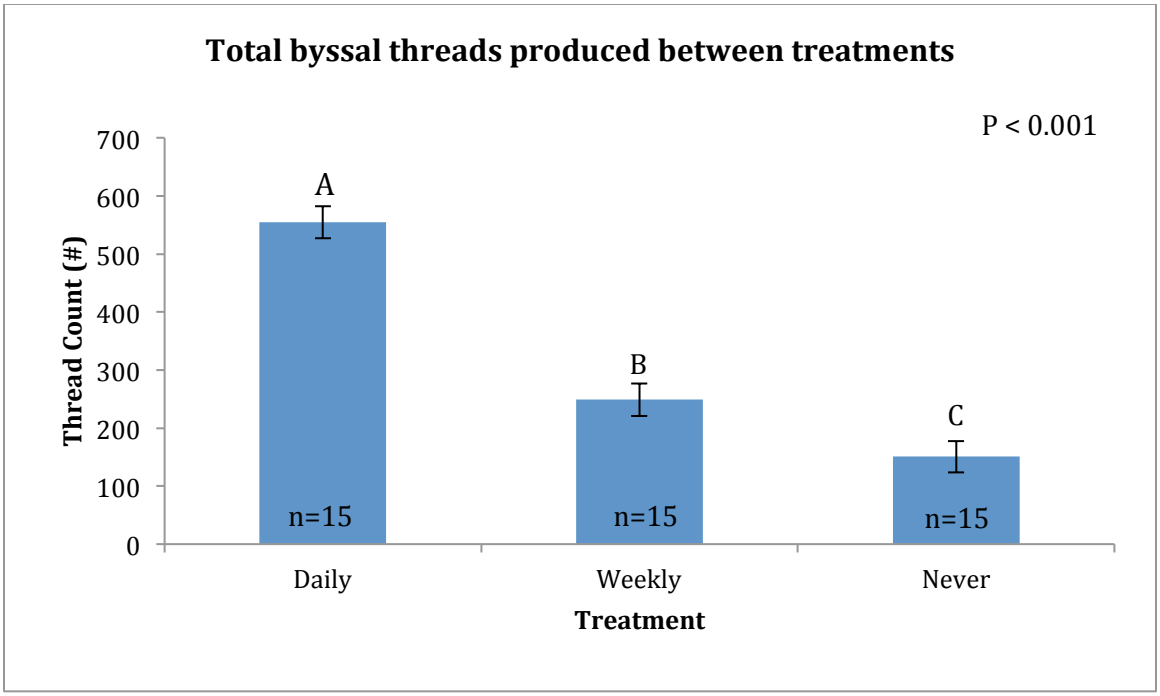

Figure 1: Bar graph representing the averaged weekly sum of byssal threads (y-axis) produced between treatments (x-axis). Production was greater on average in "Daily" treatments when compared to "Never" and "Weekly" treatments (Holm-Sidak Method, $p<0.001$). The averaged sum of threads was also greater for the "Weekly" treatment when compared to the "Never" treatment (Holm-Sidak Method, $p=0.016$). Error bars represent ±1 standard error.

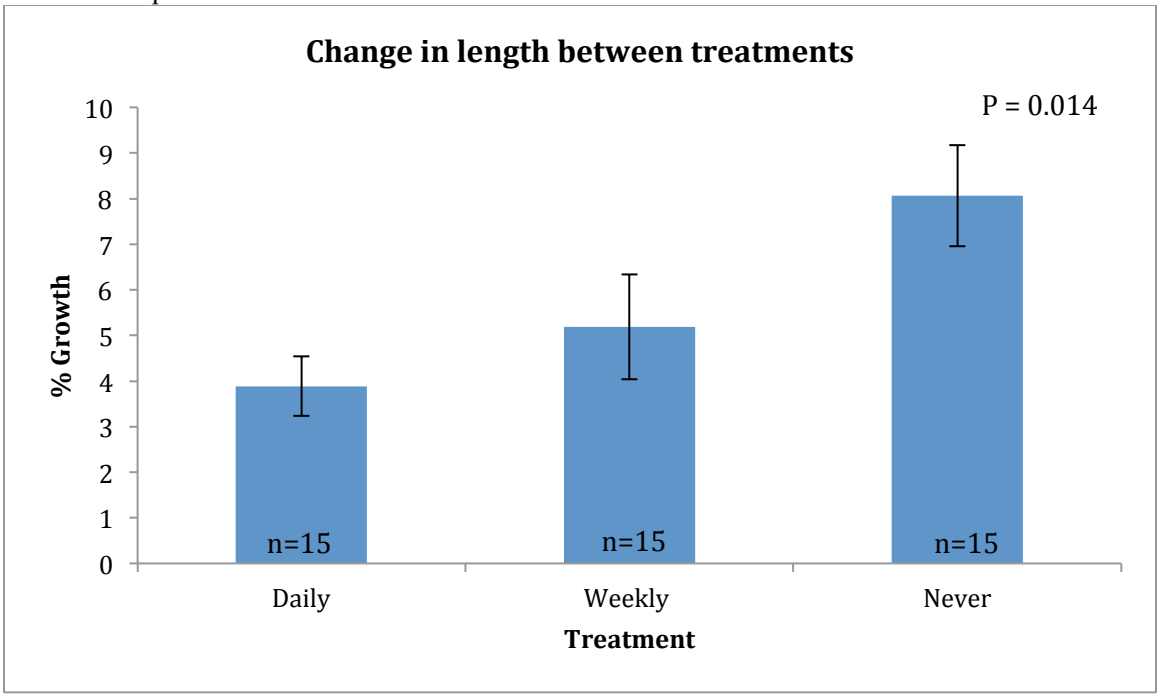

Figure 2: Bar graph representing percent change in length (y-axis) between treatments (x-axis). Changes in length were on average greater in "Never" treatment in comparison to the "Daily" treatment (Tukey Test, $p<0.050$). Error bars represent ±1 standard error.

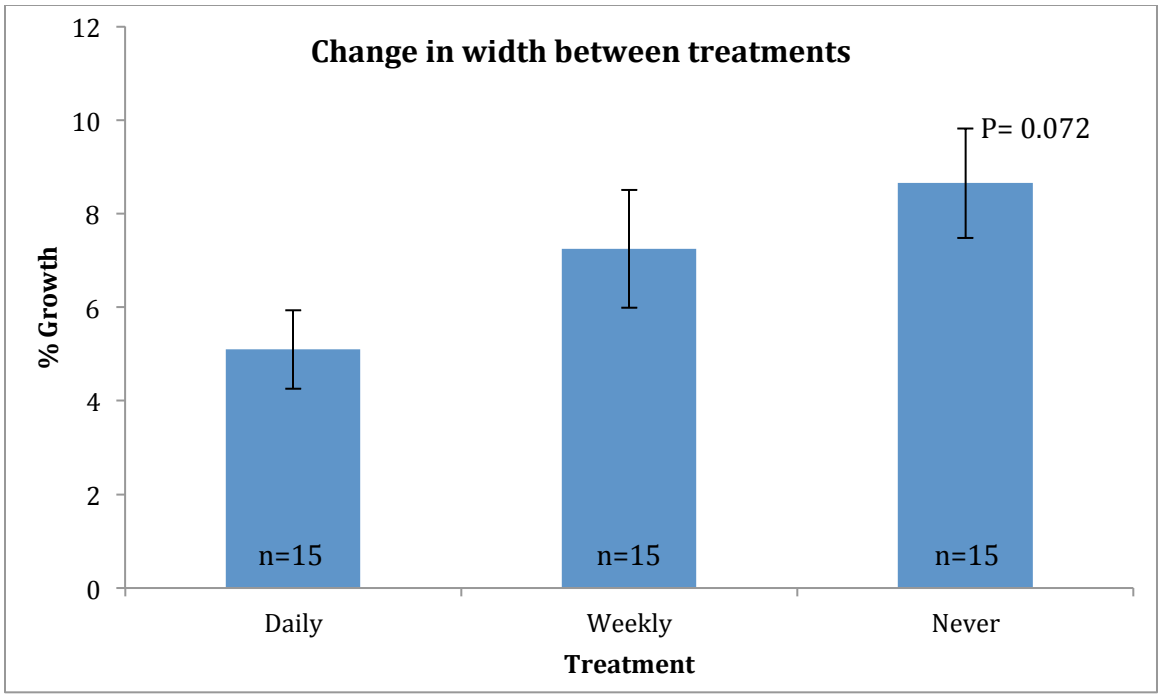

Figure 3: Bar graph representing percent change in width (y-axis) between treatments (x-axis). Changes in were higher in "Never" treatments although not significant between treatments (One-way ANOVA, p=0.072). Error bars represent ±1 standard error.

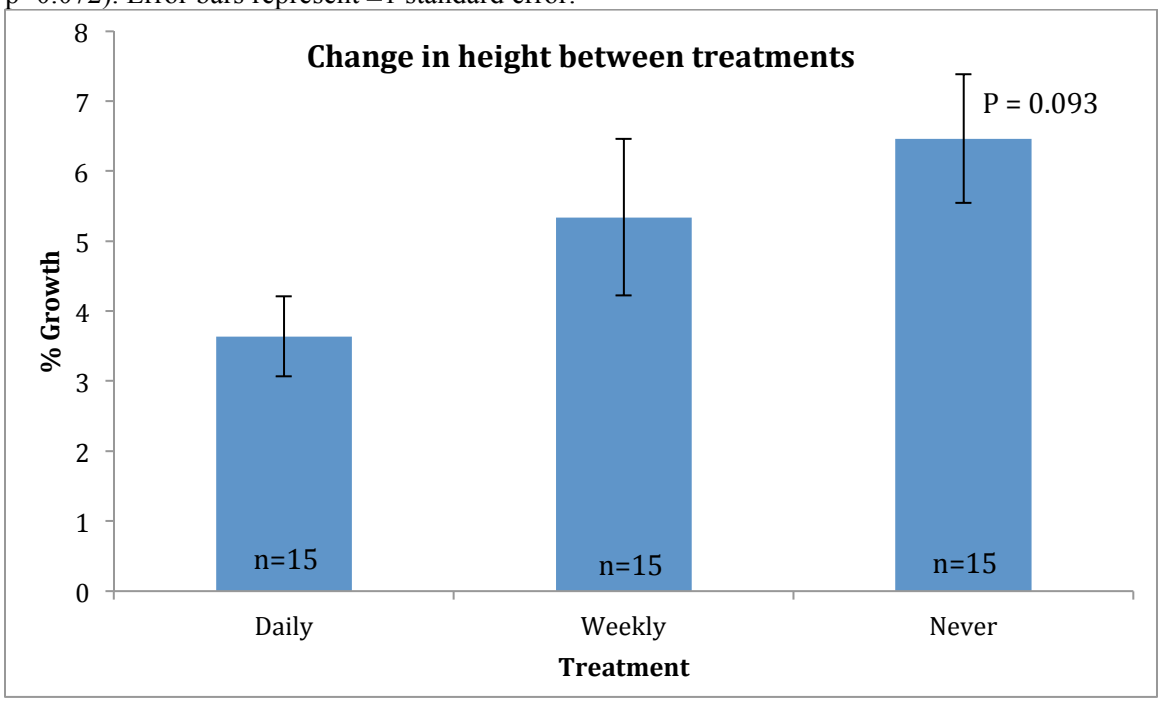

Figure 4: Bar graph representing percent change in height (y-axis) between treatments (x-axis). Changes in were higher in "Never" treatments although not significant between treatments (One-way ANOVA, p=0.093). Error bars represent ±1 standard error.

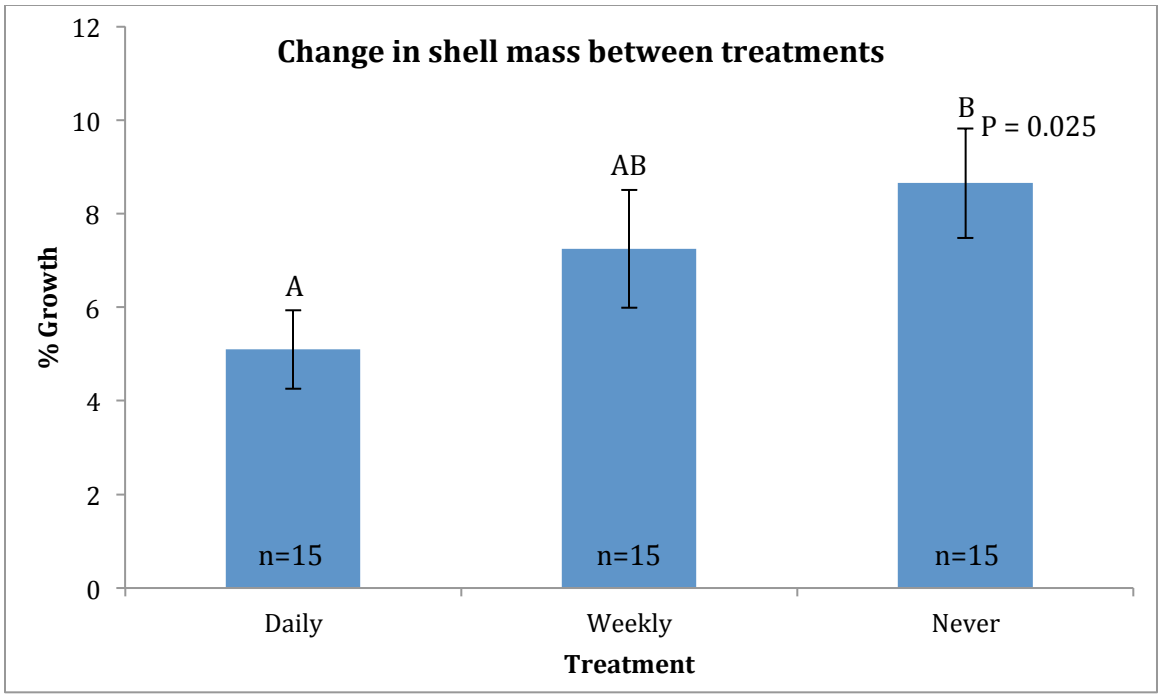

Figure 5: Bar graph representing percent change in shell mass (y-axis) between treatments (x-axis). Changes in shell mass were on average greater in "Never" treatment in comparison to the "Daily" treatment (Holm-Sidak Method, p=0.025). Error bars represent ±1 standard error.

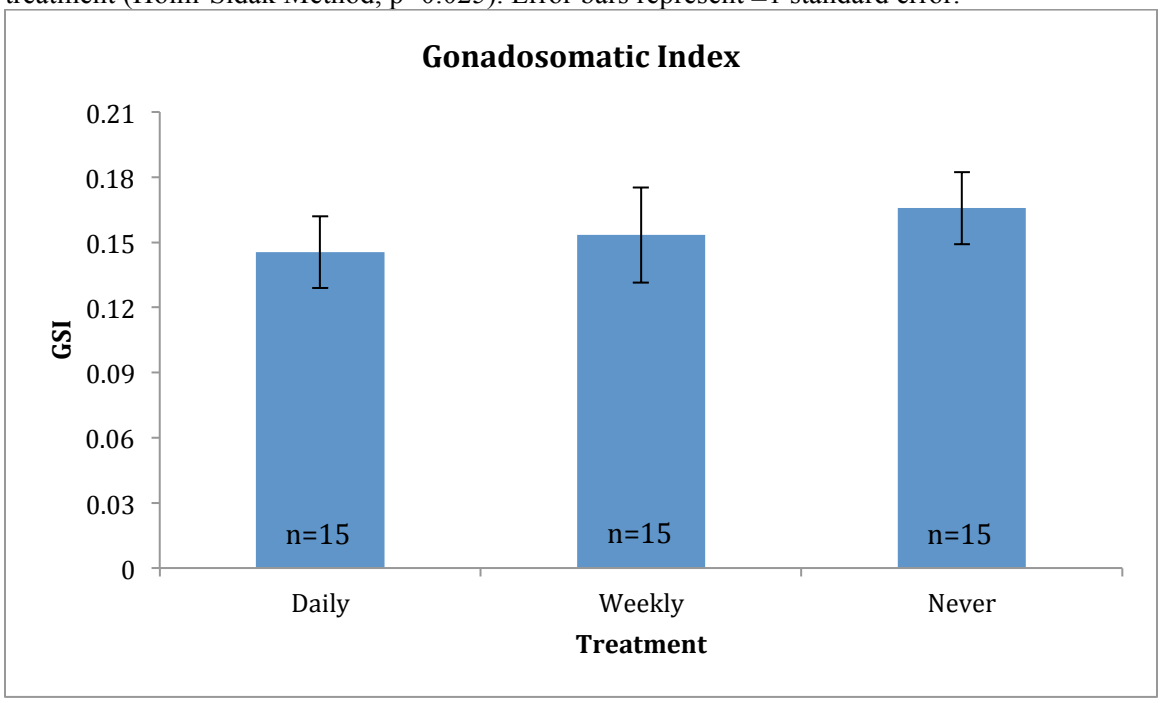

Figure 6: Bar graph representing changes in the gonadosomatic index (y-axis) between treatments (x-axis). Changes were higher in "Never" treatments although not significant between treatments (One-way ANOVA, p=0.772). Error bars represent ±1 standard error.

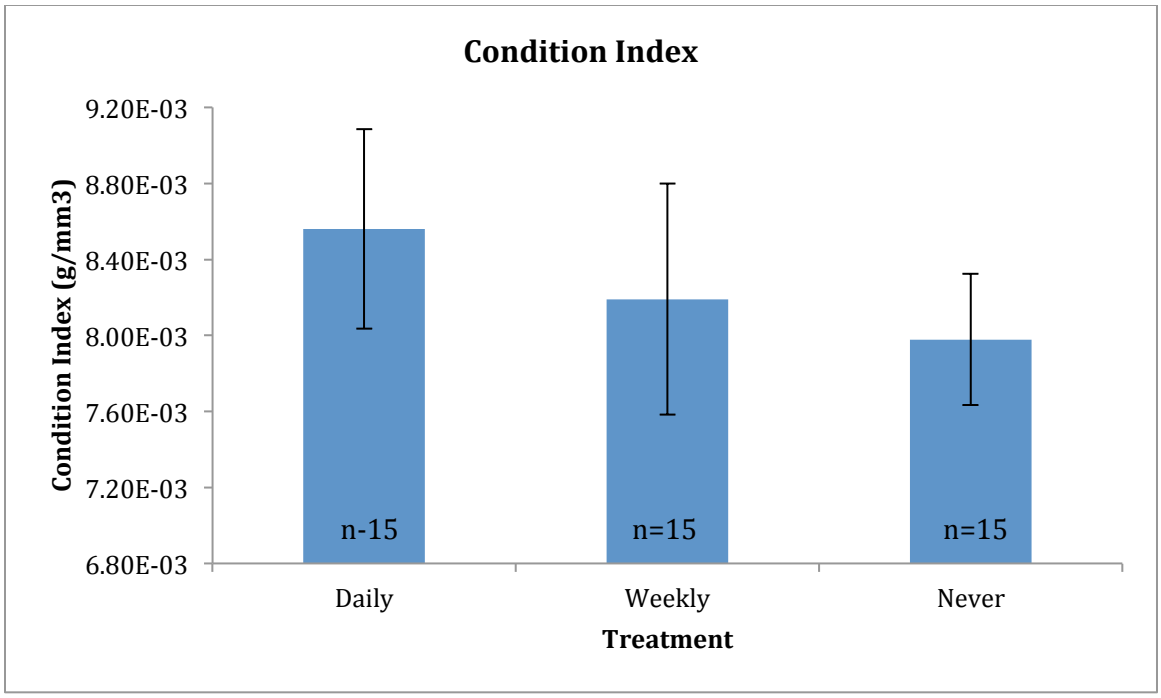

Figure 7: Bar graph representing changes in condition index (y-axis) between treatments (x-axis). Changes in were higher in "Daily" treatments although not significant between treatments (One-way ANOVA, p=0.690). Error bars represent ±1 standard error.

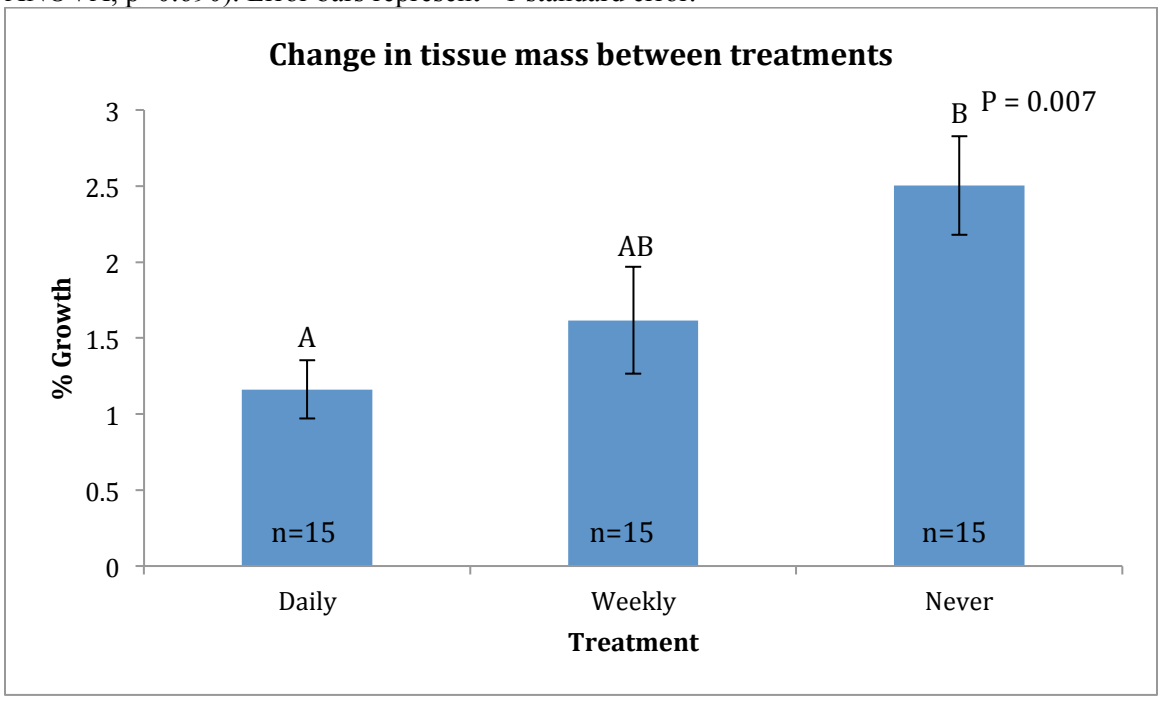

Figure 8: Bar graph representing percent change in tissue mass (y-axis) between treatments (x-axis). Changes in tissue mass were on average greater in "Never" treatment in comparison to the "Daily" treatment (Holm-Sidak Method, p=0.007). Error bars represent ±1 standard error.

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