Resource allocation to a structural biomaterial: induced production of byssal threads decreases growth of a marine mussel

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Authors' contributions

KS and EC conceived the ideas and designed the experiments;
ER and KS designed the mathematical model;
LN, MM, KH, and SL collected the data;
ER analyzed the data;
ER led the writing of the manuscript.
EC and KS contributed critically to manuscript drafts and all authors gave final approval for publication.

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1. Abstract

1. The biomechanics of specialized mechanical structures produced by organisms provides crucial fitness advantages. The energetic cost associated with producing these structural materials and the resulting energetic trade-off with growth, however, is rarely quantified. We integrate resource allocation to structural material production with an energetic framework by combining an experimental manipulation with an energetic model.

2. Mytilid bivalves produce byssus, a network of collagen-like threads that tethers individuals to hard substrate. We hypothesized that a manipulation that induces the production of more byssal threads would result in increased energetic cost and decreased growth of the species *Mytilus trossulus*.

3. In month-long field experiments in spring and autumn, we severed byssal threads across a range of frequencies (never, weekly, daily), and measured shell and tissue growth. We then quantified the costs associated with the production of byssal threads using a Scope for Growth model.

4. We found that byssal thread removal increased byssal thread production and decreased growth. The cost calculated per byssal thread was similar in the spring and autumn (~1 J/thread), but energy budget calculations differed by season, and depended on thread quantity and seasonal differences in assumptions of metabolic costs.

5. This work demonstrates that the cost of producing a structural material has a substantial effect on mussel energetic state. The energetic cost of producing byssal threads was 2-8% percent of

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the energy budget in control groups that had low byssal thread production, and increased 6 to 11-fold (up to 47%) in mussels induced to produce threads daily.

6. We propose that characterizing the trade-off between the cost of biomaterial production and growth has implications for understanding the role of trade-offs in adaptive evolution, and improved natural resource management and conservation practices.

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2. Introduction

The specialized mechanical structures organisms produce, such as cactus spines, spider webs, and bivalve shells provide a range of fitness advantages, including predator deterrence, resource acquisition and abiotic stress amelioration (Crofts and Anderson 2018, Vogel 2013, Gosline 2018). The production of a structural biomaterial, however, requires an investment of energetic resources. The investment might result in energy allocation trade-offs that shift performance traits on the individual level, and affect population dynamics (Sebens et al., 2018) and spatial distributions of organisms (biomechanical ecotype, Read and Stokes 2006). For example, the altered growth and development of plants in response to wind or mechanical perturbation (thigmorphogenesis) reduces plant size and fecundity (Telewski 1998, Chehab 2008). Similarly, the induction of dragline spider silk production reduces spider survival and fecundity (Bonte et al. 2016). For marine bivalves, a greater cost of shell production induced by low salinity conditions can affect energetic limitation (Sanders et al. 2018). In conditions of low food availability and/or high metabolic cost, such trade-offs could be greater and thus more evident; structural biomaterials would be prioritized at the expense of growth (Clarke 1999) or their production and maintenance could decline altogether (Melzner et al. 2011).

Energy budget models provide a framework for investigating energy allocation trade-offs by explicitly quantifying energetic fluxes associated with consumption of food, maintenance of cellular tissues, and growth of somatic and reproductive tissues, and reproduction. Examples of these types of models include Scope for Growth (Widdows and Bayne 1971, Thompson and Bayne 1974, Bayne et al. 1976), fish bioenergetics (Kitchell et al. 1977), and Dynamic Energy Budgets (Kooijman 2010). This mechanistic approach has been used to study relationships

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between environmental factors (e.g. energy inputs and temperature- or salinity-dependent metabolic costs) and organismal processes (soft tissue growth and reproductive output; e.g. Kooijman 2010, Kearney et al. 2010, 2012, Sarà et al. 2011, 2013, Matzelle et al. 2015, Maar et al., 2010, Maar et al. 2015). Different energy budget frameworks often yield similar biological predictions from environmental variables (e.g. Nisbet et al., 2012, Filgueira et al. 2011), but differ in complexity and in their handling of uncertainty (Boersch-Supan and Johnson 2019). Energy budget models also provide a flexible framework with which to evaluate trade-offs with structural materials since structural material production costs correlate with well-described bioenergetic fluxes (Sarà et al. 2013, Sebens et al., 2018, Sanders et al. 2018), and can have different mass-specific costs (Brody 1945, Sanders et al., 2018). SFG models provide a simple conceptual framework where tissue growth is represented as a function of consumption of food minus physiological costs (Widdows and Bayne 1971, Sebens et al., 2002, Bayne et al., 1976, Figure 1). The combination of these models with experimental manipulations of the quality (Sanders et al., 2018) or quantity of structure produced by organisms provides an excellent opportunity to study energy allocation and trade-offs.

An example of a biomaterial known to be influenced by external conditions is byssus, a structural material made by bivalves that consists of a network of collagen-like threads that tethers each animal to hard substrate (Bell and Gosline 1996, Waite et al. 1998). Marine mytilid mussels are a common organismal study system for energetic models (Van der Veer 2006, Kooijman 2010, Sebens et al., 2018), in part due to their ecological and economic importance. The mechanical strength of byssus has consequences across multiple scales of biological organization, including life history traits, mussel population dynamics, and community structure (Denny 1995, Carrington et al. 2015). For example, mussels act as ecosystem engineers (Borthagaray and Carranza 2007).when they use their byssus to densely aggregate into mussel beds a physical structures which provide refuge for associated species by limiting flow (O'Donnell 2008). Byssal thread structure facilitates culturing of this species; mussels attach to collector ropes as larvae (Brenner and Buck 2010), and as adults, form attachments to culture ropes without a surrounding net that would otherwise limit flow and increase fouling (Korringa 1976).

Previous studies estimated byssal thread production as 8 to 10% of the energy budget of mussels (Hawkins and Bayne 1985, Lurman et al. 2013). An elemental balance method

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demonstrated that ~8% of both the carbon and nitrogen incorporated into *Mytilus edulis* organic tissues was incorporated into byssal threads during a summer period of net growth (Hawkins and Bayne 1985). Lurman et al. (2013) found that respiration increases approximately 10% during periods of thread production. These findings provide estimates of the baseline cost of byssal thread production, but they do not account for the variable rate at which threads are produced or the potential energetic trade-off with other processes such as growth. The production of byssus also requires a cascade of events that include animal activity, including animal movement, foot extension, and chemotaxis of the foot to identify a suitable location to establish attachment. The quantification of carbon and nitrogen investment in byssus (~8%, Hawkins and Bayne 1985), and the instantaneous increase in respiration (Lurman et al. 2013) may therefore account for only part of the full cost of production of byssus.

The energetic trade-off between thread production and tissue growth can be characterized with a Scope for Growth model (SFG, Figure 1). Mussels modulate their production of byssal threads in response to a range of environmental conditions, such as increased wave disturbance (Van Winkle 1970, Young 1985, Lee et al. 1990, Dolmer and Svane 1994, Bell and Gosline 1997, Moeser et al. 2006, Carrington et al. 2008), seawater temperature and pH (Newcomb et al., 2019, O'Donnell et al. 2013, George et al. 2018), as well as seasonal and/or reproductive cycles in natural systems (Zardi et al., 2007, Moeser and Carrington 2006, Carrington 2002, Newcomb 2015). Within the framework of a SFG model the theoretical variable, SFG, can be used an index of tissue growth (including gonadal and somatic tissue growth, and the difference between consumption minus physiological cost; Widdows and Bayne 1971, Sebens et al., 2002, Bayne et al., 1976, Figure 1).

In natural environments, mussels modulate their production of byssal threads depending on environmental conditions, but in the laboratory the production of new byssal threads can be experimentally stimulated by severing the network of byssal threads (Young 1985). Firstly, we hypothesize that experimental manipulation (severing) of byssal threads will increase energy allocated to byssal thread production (Figure 1). We predict that mussels in treatments with greater byssal thread severing frequencies produce more byssal threads . Secondly, we hypothesize that energy allocation to byssal thread production is prioritized over tissue growth (Figure 1). Byssal threads are produced even under starvation conditions (Clarke 1999, Roberts 2019), and starvation does not reduce the production of byssal threads of larger mussels with a

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large glycogen energetic reserve (Babarro et al. 2008, 2010). We predict that mussels in treatments with greater byssal thread severing frequencies have decreased tissue growth. To test our hypotheses, we severed byssal threads at different frequencies and quantified the effect on byssal thread production and tissue growth. We combine the results of our manipulative experiments with a SFG model to evaluate how much energy mussels allocate towards byssal thread production, and away from tissue growth and reproduction, when a higher rate of production is necessary to maintain attachment. We used the growth data in a two-step optimization approach to determine the cost of producing threads by correlating tissue growth with estimated SFG across a range of thread production rates. The SFG model was then used to estimate the allocation of energy towards byssal threads relative to other costs and production across the range of quantity of structural material produced. We demonstrate an energetic trade-off of production of a structural material, mussel byssus, with growth, and show that enhanced production of mussel byssus can have a substantial metabolic cost, much higher than previous estimates.

3. Materials and Methods

3.1. Field Manipulation of Byssal Thread Production

The effect of thread severing frequency on thread production rate and growth of *Mytilus trossulus* was investigated in a field setting over 1 month in autumn 2013 (mid October to mid November) and in spring 2014 (late April to late May). *Mytilus trossulus,* ranging approximately 2-3 cm length (~80 to 200 mg dry weight tissue), were collected from Argyle Creek on San Juan Island, WA (Lat 48.521652°N and Long 123.014061°W) and transported to Friday Harbor Laboratories (Lat. 48.525350°N, Long. 123.012521°W). The pre-existing byssal threads were severed from each mussel using scissors before the mussel was placed in a flexible mesh enclosure (10 cm x 22 cm, HDPE vexar plastic, 1 cm^2 mesh size) suspended from a floating dock at ~1m depth. Seawater temperature and salinity were similar in both two month-long experiments (autumn – 9.7 ± 0.4 C, 30.3 ± 0.4 psu; spring – 9.4 ± 0.4 C, 30.6 ± 0.3 psu, means ± SD, BCO-DMO dataset, Carrington 2019, Figure S4). The three treatments differed in the frequency at which the newly produced byssal threads were severed during the experiment: never, weekly or daily (or 0, 1 and 7 times per week, respectively). Fifteen mussels were distributed evenly among three replicate enclosures for each treatment.

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Mussels were labeled with numeric tags attached to their shell with cyanoacrylic acid. They were tethered with glue inside the enclosure using ~2cm of fishing line epoxied to the shell to limit movement and provide isolation from other mussels. In the never-severed (control) treatment, mussels were attached such that they were unable to reach the cage surface with their foot, thus limiting their opportunity to attach byssus. This was done to ensure that this group of mussels achieved the lowest possible production by producing a minimum of threads. Mussels in this treatment did, however, attach byssal threads to their own shell and to their tether.

Mussels in the "daily" treatment group were monitored for byssus production every day by counting and severing newly produced byssal threads. New byssal threads were also counted and severed for mussels in the "weekly" treatment group, but at a lower frequency of once per week. New byssal threads of the mussels in the "never" treatment group were not severed and were counted at the end of the 4-week experimental period. Thread production of each individual mussel was counted in all treatments, however in the "never" control group in spring only the total number of byssal threads produced by the group was recorded. This value was divided by sample size to obtain an average thread production for each individual in this single group.

3.2. Mussel Condition, Length, and Weight Measurements

Shell growth was calculated as the change in shell length, measured with calipers (±0.1 mm). Buoyant weight (±0.001 g) was determined in seawater at the beginning and end of the experiment (salinity ~30 psu). Buoyant weight was used as a measure of total animal wet weight, including shell and tissue. Since body tissue is a similar density to seawater, this non-destructive metric is representative of changes in shell weight of individual mussels. At the end of the experiment, the mussels were sacrificed to obtain dry tissue and shell weight measurements. Specifically, gonad and somatic tissues were removed from the shell and dried at 60°C to a constant weight, and the dried shell weight was measured (±0.01 g). Condition index (CI) was calculated for each mussel by dividing dry tissue weight (g) by shell length cubed (mm^3; Crosby and Gale 1990). Gonadal-somatic index (GSI) was calculated by dividing gonad weight (g DW) by total tissue weight (g DW; Carrington 2002). Mortality during the month-long experiment was 17% in the spring and 4% in the autumn. As a result of mortality, sample sizes ranged from 11 - 15 per treatment. Two mussels in the autumn died just prior to the end of the experiment and final length was estimated from the growth rate, and tissue weight was estimated from the

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relationship between length and tissue mass of the sample population at the end of the experiment.

3.3. Energetics and Energy Allocation to Byssus

The allocation of energy towards byssus production was determined using a SFG framework following the general method of Bayne et al., (1976), with modifications suggested by Sebens et al. (2018) and Sanders et al. (2018). First, the cost per thread was calculated from the relationship between thread production and tissue growth. Then, this cost was incorporated into a SFG model and used to calculate the proportion of energy allocated to byssal thread production and the metabolic cost of byssus relative to baseline somatic costs. Model parameters are summarized in Table 2.

All energy budget calculations are expressed as daily fluxes (in J), and the calculations for each animal used normalized values from the four-week experiment. Scope for Growth (SFG, J), the energy available for growth (somatic and gonad), was calculated as follows:

$$\mathrm{SFG} = \mathrm{E} - \mathrm{cost}_{\text{non-byssus}} - \mathrm{cost}_{\text{byssus,}} \tag{1}$$

where *E* is the energy intake (J), $cost_{non\ byssus}$ is the cost of tissue maintenance (J), and $cost_{byssus}$ is the cost of producing byssus (J). We assumed mussels were minimally reproductive because mussels were small and had a low proportion of tissue that was reproductive (length < 3 cm, GSI < 0.20). Gonadal and somatic tissue maintenance costs are included in the term, $cost_{non\text{-}byssus}$ (Eq. 1). Most of the gonad weight consisted of structural tissues, rather than gametes, in these small mussels and thus we did not calculate a separate allocation or cost for gamete production.

Individual energy intake (E) depends on initial tissue mass ($TM_{initial}$, mg DW):

$$\mathrm{E} = \mathrm{f} \times \mathrm{a'} \times \mathrm{TM}_{\mathrm{initial}}{}^{\mathrm{d}}, \tag{2}$$

where *f* is the relative food availability coefficient (unitless), *a'* is the energy intake coefficient (J/mg^d) and is described in more detail in equation 4, and *d* is the energy intake exponent (unitless). The relative food availability coefficient (*f*) is a scaling factor for the amount of food available during the experiment and was estimated from the experimental data for each season. Food availability was considered equal for all mussels within each season since they were exposed to the same water mass. The energy intake exponent (*d*) is an allometric scaling factor for the relationship between tissue mass and gill area (the food capture surface for mussels) and has been well described for *M. edulis* (Jones et al. 1992; Bayne and Newell 1983, Table 2).

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The metabolic cost of somatic and gonadal tissue for each experimental mussel was calculated as a function of initial tissue mass, $TM_{initial}$ (mg DW):

$$\text{cost}_{\text{non-byssus}} = \text{b} \times \text{TM}_{\text{initial}}^{\text{e}}, \tag{3}$$

where b is the mass-specific metabolic cost coefficient (J/mg^{e}), and e is the allometric cost exponent (unitless) that relates mass-specific metabolic cost and tissue mass. We assume that the cost relates directly to the amount of tissue ($e = 1$, Bayne et al., 1976), a value that has been shown to be well-conserved among bivalve species (Sarà et al. 2013, Kooijman 2010), thus b has units of J/mg. *Mytilus spp.* respiration per unit tissue mass generally differs by season and follows reproductive cycles (Widdows 1978), so b was determined from the spring and autumn measurements of mass-specific oxygen consumption of Fly and Hilbish (2013) for *M. trossulus* from WA. Respiration at 10°C was estimated from a linear regression of the respiration measurements from 5°C to 20°C, and the standard error was estimated as the average standard error from each temperature: 0.170 ± 0.040 ml O_2 / (hr × g DW) in autumn and 0.333 ± 0.089 ml O_2 / (hr × g DW) in spring (Table 2). The spring and autumn values were then converted to daily values to yield the metabolic cost coefficient (b) for autumn (0.81 ± 0.019 J/mg) and spring (0.158 ± 0.043 J/mg; Table 2, Riisgård and Randløv 1981).

The energy intake coefficient (a') was calculated as the average amount of food available over the course of the lifespan of a mussel to produce an individual of a given size, given optimal size theory (Table 2; Sebens 2002):

$$\text{a}' = \frac{\text{b} \times \text{e}}{\text{W}_{\text{opt}}^{\text{d-e}} \times \text{d}}, \tag{4}$$

where W_{opt} is the energetic optimal size (mg DW), or the size at which the difference betw intake and costs is maximized and the coefficients b, d and e are defined in equations 2 and 3. By using this metric for the intake coefficient, we assume that mussels at their maximal size have a maximal surplus and that all surplus goes to reproduction rather than to growth. We assume a value of 720 ± 60 mg DW for W_{opt} (Roberts 2019; Table 2). Note that the seasonal difference in respiration resulted in a reduced different metabolic cost coefficient and a greater energy intake coefficient in spring compared to autumn (b and a', Table 2).

The cost of byssal thread production was calculated as the number of threads produced, N_{Th}, multiplied by the cost of each individual thread, h (J/thread):

$$\text{cost}_{\text{byssus}} = \text{h} \times \text{N}_{\text{Th}}.$$

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(5)

Substituting the equations for intake (Eq. 2), non-byssus cost (Eq. 3), and byssus cost (Eq. 5) into Eq. 1 yields the following equation for SFG as a function of initial tissue mass and byssal thread production:

$$\mathrm{SFG} = \mathrm{f} \times a' \times \mathrm{TM_{initial}}^{\mathrm{d}} - \mathrm{b} \times \mathrm{TM_{initial}}^{\mathrm{e}} - \mathrm{h} \times \mathrm{N_{Th}}. \tag{6}$$

This SFG model was then fit to the experimental tissue growth measurements using the optimization method, described in the following sections.

3.4. Tissue Growth Calculation

Mussel tissue growth was calculated as the difference between final and initial tissue dry weight, where final dry weight was measured directly and initial dry weight was estimated (because direct measurement is destructive). Specifically, an estimate of initial tissue mass ($TM_{initial\_fit}$, mg DW) was calculated from shell length ($length_{initial}$, cm) as follows:

$$\mathrm{TM_{initial\_fit}} = \delta \times \mathrm{length_{initial}}^{3}\,, \tag{7}$$

where δ is the shape coefficient (mg DW/cm^3) that relates length and tissue mass and was estimated from final length and tissue weight for each season (Table 2). We assumed that the exponent relating length and tissue mass is 3 (Kooijman 2010) and confirmed this assumption with a separate sampling (see Appendix S1 in Supporting Information). The residuals of the final tissue mass ($TM_{residual}$, mg DW) were calculated as the difference between the measured final tissue mass (TM_{final}) and the final tissue mass value estimated from the shell length:

$$TM_{residual} = \mathrm{TM_{final}} - \delta \times \mathrm{length_{final}}^{3} \tag{8}$$

The residuals ($TM_{residual}$, mg DW) were added to the estimate of the initial tissue mass ($TM_{initial\_fit}$):

$$\mathrm{TM_{initial\_g}} = \mathrm{TM_{initial\_fit}} + \mathrm{TM_{residual}} \tag{9}$$

An additional independently-determined value of initial dry tissue weight ($TM_{initial}$, was calculated from the relationship between dry tissue weight and buoyant weight in each season. This initial tissue mass estimate was used in the equations for intake and cost (Eq. 2, 3, and 6).

3.5. The Cost of Byssus Estimation Using a Two-Step Optimization

We used the experimental data to calculate two parameters, *h* (cost per thread, J/thread) and *f* (food scalar, unitless), using a sequential linear regression. This optimization method

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minimized the difference between the measured tissue growth rate (G_{TM}, joules/day) and predicted tissue growth rate calculated as SFG from the initial tissue mass ($TM_{initial}$) and thread production (N_{Th}) for each individual, and allowed for an estimate of population error from the data.

Step 1 of the two-step optimization was a calculation of the cost per thread from the relationship between growth and thread production. If the production of N_{Th} byssal threads decreases growth, then the cost of thread production can be calculated from the slope of this relationship. In other words, energy that would have been used for growth had to be diverted to production of N_{Th} byssal threads. Specifically, the cost per thread (*h*, joules/thread) was estimated as the magnitude of the coefficient B_1 in a linear regression relating thread production (N_{Th}, threads/day) and tissue growth (G_{TM}, joules/day):

$$G_{TM} = \mathrm{B}_0 + \mathrm{B}_1\,\mathrm{x} + \epsilon, \tag{10}$$

where x is the rate of thread production (N_{Th}, threads/day), the intercept, B_0, is excess, unaccounted for energy, and ϵ is a random noise variable.

Step 2 of the two-step optimization estimated relative food availability (*f*, unitless) as the coefficient B_1 from a linear equation (Eq. 10), where B_1 multiplied by x is now the intake (E, J/day), and the intercept, B_0, is the negative sum of byssal thread cost ($cost_{byssus}$, J/day) and metabolic cost ($cost_{non\text{-}byssus}$, J/day) for each individual mussel. In this step, the intercept, B_0 is calculated from costs and is not estimated from the linear regression. ϵ remains as the random noise variable relating the predicted and observed growth values for each individual.

The proportion of the energy budget allocated to byssus, (*proportion of cost*, unitless) was then calculated by dividing $cost_{byssus}$ by E for each individual mussel:

$$\text{proportion of cost} = \frac{\text{cost}_{\text{byssus}}}{\text{cost}_{\text{byssus}} + (\text{cost}_{\text{non-byssus}} \times \text{P}_{\text{Som}})}, \tag{11}$$

where P_{Som} is the proportion of the total tissue that is somatic tissue. A proportion of cost of 1 indicates that all costs are byssus costs, and proportion of cost of 0.5 indicates that byssus and somatic costs are equivalent.

3.6. Statistical Analysis

All statistical analyses and model calculations were performed with R software for Mac OSX (version 3.4, R Core Team, 2017). Data were transformed to normalize distributions; thread

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production (count data) was square root transformed, gonad index (proportional data) was arcsine square root transformed, and shell growth, buoyant weight change, tissue growth, and condition index (all continuous data) were log-transformed for statistical analyses. All transformed data met assumptions of equal variances, with the exception of tissue growth. For this metric variance differed among seasons but not within each season, limiting comparisons between seasons. The effect of experimental byssal thread manipulation on thread production, shell length and weight, tissue growth, gonad index, and condition index were evaluated for autumn and spring separately using one-way ANOVAs with thread severing frequency as fixed factor. We used two separate one-way ANOVAs for each season as a more conservative approach where we evaluated the experiments as two repeated manipulations that spanned the two seasons rather than evaluate differences between the two seasons. Additional statistical analyses that evaluated model assumptions about organismal traits are described in the supplementary methods. These are the effect of initial tissue mass and byssal thread production on tissue growth (multiple regression), and the relationship between tissue growth and byssal thread production with treatment as a fixed factor (ANCOVA). If any significant effects were present, a post-hoc Tukey test was performed to evaluate differences between groups. Preliminary analyses of the effect of byssal thread manipulation on the metrics listed above were performed using linear mixed models (LME; Zuur et al. 2009), and these analyses confirmed that the random effect of the enclosure was not significant so we did not include this random effect in further analyses(data not shown).

We ran the model parameter estimations as linear regressions in R where each parameter was estimated as the coefficient in a linear equation of all individual mussel samples within each season, separately (Eq. 10, Methods Section 3.5). Model sensitivity to the parameters used to estimate the cost of producing byssal threads and the proportion of the energy budget used for thread production for each season was determined with an individual parameter perturbation (IPP) analysis (Kitchell et al. 1977) using the estimated standard error for each parameter. A sensitivity of 1.1 indicates that a change in parameter by 1 SE causes a resultant change in simulated cost by 10%. We used parameter standard error in place of a nominal 10% change in each parameter to perturb the model in order to simulate a more realistic range of parameter values.

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4. Results

4.1. Field Manipulation of Byssal Thread Production

Byssus severing increased thread production by a factor of five in both seasons (Figure 2, Table 1). The effect of greater byssus severing frequency significantly decreased shell growth by 50% in the autumn and 25% in spring ($p < 0.001$, and $p = 0.01$, respectively; Figure 2, Table 1). Byssus severing frequency significantly decreased buoyant weight growth in the autumn, but this effect was only marginal in the spring ($p = 0.002$ and $p = 0.11$, respectively; Figure 2, Table 1). Byssus severing frequency significantly decreased tissue growth by 70% in autumn and 45% in the spring ($p < 0.001$ and $p = 0.01$, respectively; Figure 2, Table 1). GSI was overall 30% greater in the spring compared to the autumn, but there was no significant effect of byssus severing frequency on gonad index for either season ($p = 0.7$ to 0.9; Figure 2, Table 1), probably because gonad tissue was a small proportion. Condition index did not differ significantly among treatments ($p = 0.2$ to 0.7; Figure 2, Table 1). Since none of the treatments resulted in mass loss, the relationship of shell to tissue did not change radically.

There was a significant negative relationship between thread production and tissue growth across all treatment groups in both autumn and spring ($p = 0.02$ and $p = 0.3$, respectively; Table S2), but no significant relationship between initial mass and tissue growth for either season ($p = 0.43 - 0.67$; Table S2), and no interaction between these two effects on tissue growth ($p = 0.21 - 0.88$; Table S2). While there was an overall negative relationship between thread production and tissue growth, this effect was driven by the byssus severing manipulation. The range of growth and thread production was greatest in the autumn, and in this season there was a negative effect of byssus severing frequency on growth ($p = 0.007$; Figure S1, Table S3), but within each treatment an overall positive relationship between byssal thread production and growth ($p = 0.04$; Figure S1, Table S3). In the spring, there was a similar trend but both effects were only marginally significant.

4.2. Cost of Byssal Thread Production

Tissue growth predicted by the model had a smaller range (15-42 mg DW) than observed growth (10-80 mg DW) in autumn and spring, and at least 90% of the predicted growth rates had a percent error relative to observed growth of less than 40% (Figure 3, Figure S3E, F). The cost of byssal thread production was similar between the two seasons, ranging 1.0 – 1.2 J per thread

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(Table 3). Relative food availability (f, unitless) was 40% higher in the autumn than in the spring (Table 3).

The mussel energy budget components (intake, somatic cost, gonadal cost, cost of byssus and growth), as determined by the two-step optimization, are represented across the range of byssal thread production rates for each season in Figure 4. The daily cost of byssus production was proportional to the number of threads produced, and the predicted growth rate decreased as thread production increased, as observed in our experiments.

Metabolic costs of somatic and gonadal tissue were 2 times greater in spring than in autumn (Table 2), and the proportion of the energy budget allocated towards byssal thread production was 2-4 times greater in the autumn (Figure 4, Table 4). The proportion of the energy budget allocated towards thread production for mussels induced to produce threads daily was 6-11 times greater than the control group (up to 47%, Table 4). Mussels induced to produce threads daily also had a greater 'ramping up' of metabolism such that byssal costs were a greater percentage of total non-reproductive cost (41% - 66%) than the control group (6% - 24 %, Table 4). The relationship between thread production and the proportion of non-reproductive costs going to thread production was nonlinear (Figure 5, Table S4). At greater thread production rates, the proportion of non-reproductive costs approached an asymptote of 50-70%, and the byssal thread production rate that resulted in the half maximum cost was 6-8 threads per day, depending on the season (Figure 5, Table S4).

4.3. Model Sensitivity Analysis

The individual parameter perturbation (IPP) analysis demonstrated that increasing the energetic conversion factor (C.F.) by one SE increased the cost per thread (h) estimate by 10%, and the error introduced by variability in the data was greater than the error introduced by the SE of the conversion factor (C.F.) in both seasons (36% in autumn, 34% in spring; Figure S2). The cost per byssal thread was independent of the parameter values of b and d (Figure S2, Eq 10).

Both of the byssus energy allocation metrics, the proportion of the energy budget allocated towards thread production and the proportion of cost allocated towards byssus (excluding reproductive costs), were sensitive to changes to b and C.F., and neither measure was more than marginally sensitive to d. The population error of the proportion of cost allocated towards byssus also differed by treatment and was greatest for the control group that was never

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severed in the autumn (Figure S2). The error introduced by changing *b* by one SE was often similar to the magnitude of the population standard error (Figure S2). W_{opt} had no effect on these three model outputs in either season.

5. Discussion

5.1. The cost of byssus as a component of a SFG framework

The two month-long experiments demonstrated that clipping byssal threads greatly increased byssus production and significantly decreased growth. This trade-off is consistent with reports of constitutive byssal thread production regardless of growth rate or energetic input (Clarke 1999, Roberts 2019, Hawkins and Bayne 1985), depending on mussel size or glycogen reserve depletion (Babarro et al. 2008, 2010). This result supports the concept that energy allocation is prioritized towards production of byssal threads over growth (Clarke 1999), and that this trade-off is a fitness strategy that minimizes the risk of dislodgement and can maximize overall fitness (Sebens et al. 2018). Mussels that allocate too little energy to byssus production face an increased risk of dislodgement and mortality, those that allocate too much energy experience reduced growth and reproduction. Determining the optimum allocation requires a model that estimates population increase based on changes in life history, energy allocation, and environmental conditions (Carrington et al. 2015).

Using this demonstrated trade-off between byssus production and growth, we were able to quantify the energetic costs associated with producing byssus (~1 J/thread). Mussels in the control group, where byssus was severed only once at the start of the experiment, produced fewer threads, and allocated 2% to 8% of the energy budget towards threads. These results are consistent with previous estimates of up to 8% of each of the carbon and nitrogen budgets (Hawkins and Bayne 1985), and consistent with an approximate 10% increase of respiration reported during periods of thread production (Lurman et al. 2013). In contrast, severing byssus daily stimulated byssal thread production and increased energy allocation to byssus 6 to 11-fold, such that the byssus represented 41% to 66% of the total non-reproductive energetic costs.

Baseline byssal thread production rates measured in this experiment were likely lower than in rocky shore habitats. The experimental mussels were within a protected enclosure under a dock, without predators or wave forces, but were flushed by currents. Additionally, mussels that had the byssus severed at the lowest frequency (“never”) were also tethered away from substrate

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to minimize byssal thread production. In natural wave-swept environments greater hydrodynamic forces induce mussels to produce more byssus (Van Winkle 1970, Young 1985, Lee et al. 1990, Dolmer and Svane 1994, Bell and Gosline 1997, Moeser et al. 2006, Carrington et al. 2008), and high tide-pool temperatures can induce mussels to move to another location by sloughing off previous threads and producing more threads (Schneider et al. 2005). Predator cues can also induce thread production (*Mytilus edulis* - Garner and Litvaitis 2013, *Ischadium recurvum* - Brown et al., 2011), and byssal thread production is greater at sites with high predation than at those with low predation risk (Leonard et al., 1999). Unsuccessful predation might also cause thread breakage, and thus increase the production rate of new threads. Conditions that cause or require greater thread production can increase the cost of byssus to values high enough to equal the entire energy surplus (i.e. >50%) and limit growth or reproduction entirely. On the other hand, stressful conditions that limit the production of threads, such as low pH conditions where mussels remain closed, might limit investment in thread production (George et al. 2019).

A lower proportion of energy was allocated towards byssus in spring than in autumn, reflecting both a lower byssal thread production rate and ~2 times greater mass-specific respiration costs in this season (Fly and Hilbish, 2013). *M. trossulus* matures and spawns in spring (Skidmore 1983) and periods of spawning can decrease thread production (Babarro et al., 2010); byssus attachment strength decreases following seasonal reproductive periods (Zardi et al., 2007, Carrington 2002). Greater spring mass-specific respiration costs likely reflect greater reproductive costs; For the congener species, *M. edulis*, mass-specific respiration costs are ~2 times greater in the spring, corresponding with an increased reproductive status (Widdows 1978).

Overall, increased severing frequency caused a significant decrease in growth (Table 1), and, overall, there was a trade-off between byssal thread production and growth when treatments were pooled (Table S2).. After accounting for the effect of experimental treatment on growth, however, there was a significant positive relationship between thread production and growth in autumn but not in spring (Figure S1, Table S3). Within the same experimental population, variability in growth among individuals can depend on intrinsic genetic variance in growth trajectories (Dmitriew 2011), size-specific intake and metabolic costs (Martin et al. 2012), and extrinsic factors such as microscale differences in flow and food availability (Denny and Gaylord 2010). Although all mussels in these experiments were exposed to the same food concentration

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(within a season), genetic variation in food uptake (Dmitriew 2011), individual mussel behavior (gape, closure, pumping; Miller and Dowd 2019), and the location of mussels in the cages might differ. The resulting variability in food intake could account for a range of growth rates among individuals in this study. On one hand, these results support the hypothesis that both thread production and growth could be positively correlated across a broad range of energetic surplus, if individuals with increased growth also have greater resources with which to produce byssal threads (Roberts 2019). On the other hand, however, when a large proportion of the energy budget is allocated towards byssal thread production, in this case induced by a greater severing frequency, there is a strong negative trade-off.

5.2. Model sensitivity analysis and model limitations

Traditionally, sensitivity analyses (i.e. individual parameter perturbation, or IPP) have been used to characterize the sensitivity of model results to a nominal change (i.e. 10%) in parameter values (Monaco et al. 2014, Sanders et al. 2018, Kitchell et al. 1977). Our sensitivity analysis compared population error due to variability among individuals to the influence of the error introduced by uncertainty in parameter values. The cost per byssal thread calculation was sensitive to the energy conversion factor (*C.F.*, J/mg DW; Figure S2), the energy required to produce one unit of tissue mass. The value used for this parameter was consistent with Scope for Growth methodology (e.g. Sanders et al. 2018, caloric density of tissue), but this value differs depending on the bioenergetics theory employed (Kooijman 2010, Rumohr et al. 1987, Table S1). A lower caloric density of the tissue would decrease the magnitude of the calculated energy budget and magnitude of the individual thread costs (Figure S2). The energy required to produce tissue mass includes both the overhead energy consumed in anabolism and catabolism as well as the cost (stored energy) of the building blocks of mass in the organism. We used the simplifying assumption that the energy required for growth is proportional to the change in mass and that mass and energy can thus be interconverted (e.g. DEB theory -Kooijman 2010), but ultimately both energy and mass are required for growth. Further, mussel shell calcification is estimated to range between 30-60% of the energy budget for Baltic *M. trossulus* with the greatest cost at lower salinities (6 – 16 psu, Sanders et al. 2018). Salinities remained high (~30 psu) during this experiment, and thus calcification costs may be lower than estimated by Sanders et al., (2018). Previously, the cost of shell has been attributed solely to the cost of producing shell organic

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matrix. Not accounting for energy expenditure to the production of inorganic substance is a limitation of many energy budget models. Similarly, SFG models that do not account for the cost of byssus, may overestimate the fractional contributions of other components of the organism relative to all energy assimilated (e.g. shell). The utility of a simple model, however, in answering a specific research question should not be minimized, especially when contributions of overhead costs are not known with much certainty.

Uncertainty in respiration and the resulting metabolic cost coefficient, *b*, contributed substantially to our uncertainty of our calculation of the proportion of the energy budget allocated towards thread production (Figure S2, Table 2). Respiration is variable even within individuals of the same population at the same temperature (Fly and Hilbish 2013, Sanders et al. 2018), suggesting that the contribution of the uncertainty of respiration to energy budget calculations should be carefully considered (Boersch-Supan and Johnson 2019). We used published respiration values for *M. trossulus* in the same season from the same site estimated for the environmental seawater temperature using a linear fit (10°C, Fly and Hilbish 2013, Figure S4). These published respiration values were for smaller mussels than those in our experiments, so respiration values were scaled according to size (spring 0.25g, autumn 0.47g Fly and Hilbish 2013, Table 2). We make the simplifying assumption that the cost of threads is not included in published respiration values (Fly and Hilbish 2013), though thread production and feeding may increase respiration (Lurman 2010). We assumed that respiration scaled linearly with tissue mass ($e = 1$), based on the theory that maintenance costs scale with the volume of the individual (Kooijman 2010, Sarà et al. 2013), but given empirical evidence from other organisms (Metabolic Theory of Ecology; Brown 2004), this exponent is likely less than 1 (0.75 for *M. edulis*, Widdows, 1987). This model evaluated a 'snapshot' of growth for one size class (2-3 cm, juvenile mussels) over just one month. Within this small size range, we observed no significant relationship between mass and the actual observed growth (Figure 3, Table S2) but a positive relationship between the mass and predicted scope for growth (Figure S3). Investigations that include a wider range of organism sizes may more fully capture the relationship between SFG and mass for this species. Moreover, size can act as a confounding factor under conditions of stress, and it is possible that size could have affected the trade-off between size and thread production even within the small size range of our experiment. Experiments with a wider range of sizes would give a clearer picture of how an energetic trade-off to byssus is affected by mussel

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size. Smaller juvenile mussels can produce a greater number of threads than larger adults (30 vs. 90 mm *M. galloprovincialis*, Babarro et al. 2008), so adult mussels might have a lower energetic investment in thread production. Further, experiments performed over the longer-term (>1 month) could elucidate the effect of byssus severing on mussel condition, which might reflect unequal energy allocation to volumetric size and tissue mass.

According to our model, intake (*E*) was 33% greater in spring than autumn, reflecting the magnitude of *f* multiplied by *a'*. In spring, the greater intake counteracted greater mass-specific respiration costs when compared to autumn experiments (Fly and Hilbish 2013). Phytoplankton blooms often occur in the spring in the Salish Sea (Murray et al. 2015, Lowe et al. 2016). The congener species, *M. edulis*, depends on a nutrient reserve during and after spawning (Gabbott 1976), and across U.S. East Coast latitudinal gradients, spawning corresponds with the timing of greater nutrition for adults and larvae, rather than temperature cues (Newell et al. 1982). In bioenergetics models, the relative food availability, *f*, is typically estimated for each site from the data and site differences are attributed to differing food quality (DEB, Kooijman 2010). Our energetics model demonstrates that if parameters (e.g. metabolic cost, the shape coefficient) are not temporally or spatially explicit (e.g. measured for each season and/or population), the explanatory power of the model may be limited if it does not account for these differences (non-stationarity; Monaco et al. 2018, 2019). We demonstrated a use of optimal size theory to calculate a scalar for lifetime average intake, a', representing lifetime average food consumption necessary to arrive at an asymptotic (maximal) size typical for the environment they were grown in (Sebens 1982, 1987, 2002). The value obtained for a' differed by season, reflecting different assumptions about lifetime metabolic costs, given differing measurements of respiration, in each season (Sebens 1982, 1987, 2002). In other words, to achieve a specific asymptotic size, the average value of a' during growth to that size can be calculated even when actual food availability is not known.

5.3. Consequences in rocky shore systems and mussel aquaculture

An understanding of the energetics of byssal thread attachment has potential consequences in rocky shore systems, mussel aquaculture, and in how we conceptualize the effects of climate change on mussels. Our findings of a trade-off between byssal thread production and growth suggests that dynamic changes in byssal thread production may impact

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mussel condition and growth in the field, consistent with the reciprocal transplant studies Babarro and Carrington (2011) with *M. galloprovincialis*. Decreased SFG might be a disadvantage if mussels do not grow fast enough to escape predation, if feeding or energetic reserves are limited by size, or if mussel energetic investment in reproduction is limited. There may be an advantage to staying small, however, when flow forces are limiting. Small mussels experience lower drag forces, and mussels that stay small could have a lower risk of becoming dislodged (Bell and Gosline 1994). Further, in exposed environments there are often fewer predators that might selectively consume smaller prey, and so it is possible that size escape from predation may be less necessary for survival in more wave-exposed environments. The formation of aggregated mussel beds may decrease hydrodynamics forces on a local scale (Moeser and Carrington 2008), and solitary mussels can produce more threads than those in an aggregation (Bell and Gosline 1997, Carrington and Moeser 2008). Greater mussel bed density may also decrease food availability and feeding (Frechette et al., 1992), and increase the likelihood of low pH and DO conditions within an aggregation of mussels (George et al. 2019), suggesting there are ecological trade-offs to forming densely aggregated mussel beds that may mirror these organismal physiological trade-offs.

An understanding of the energetics of byssal thread attachment also has implications for mussel aquaculture practices. Mussels grown in suspension culture are often redistributed, or "re-socked," to reduce line density and competition for food (Korringa 1976, Gosling 1992). This practice presents a trade-off between inducing greater byssus production costs, with potentially more food, and either increased or decreased growth or survival (Roberts 2019). Energetic limitations can thus inform mussel culture practices; redistribution of *M. trossulus* might be more successful in seasons with reduced mass-specific respiration and reproductive costs (i.e. not during the spring), or prior to stressful periods when costs are high, either due to reproduction (spring) or due to microscale low pH and DO due to respiration within mussel aggregations (late summer, George et al., 2019).

Mytilus spp. occur in coastal ecosystems and aquaculture farms globally (Gosling 1992) and thus a promising direction for future work is to evaluate physiological trade-offs of byssal thread production costs in the context of climate change. Our expanded framework of organismal energy allocation, inclusive of byssus costs, may be used to develop new hypotheses of cascading effects of local and global anthropogenic changes on organismal processes, growth,

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reproduction, and species distributions (SFG - Fly et al., 2015). On the US west coast, buoy observations indicate that wave heights have increased 0.03 m yr^{-1} (Allan and Komar 2006). Climate change is expected to increase U.S. west coast storm surge (Cheng et al. 2015) and wave heights in high-latitude coastal ecosystems around the globe (Semedo et al. 2013), which might directly lead to dislodgement and/or increase byssal thread costs and decrease growth. In our study region, ocean-estuarine circulation models predict ocean warming and acidification will be +1.5C, pH -0.18, in year 2095 relative to year 2000 (Salish Sea, RCP8.5 scenario; Khangaonkar et al. 2019). Local pH and oxygen conditions within mussel conglomerates experience intermittent declines in summer to levels that strongly affect byssal thread production and attachment strength (pH of 5, George et al. 2019). Under these conditions, mussels stay can closed for multiple days, limiting byssal thread production (George et al. 2019). In the short-term, greater seawater temperatures may decrease thread production (Newcomb et al. 2019), potentially affecting byssus cost and SFG, but longer-term exposure to greater temperatures may not affect the number of byssal threads produced (Roberts 2019).

Our work also suggests that a dynamic cost of byssus may compound or counteract the effects of climate change on intake or non-byssus costs. Warming in this region will likely decrease energetic resources available for growth for *M. trossulus* (Roberts 2019), by lowering intake rates and increasing metabolic costs for this species (Fly and Hilbish 2013). In contrast, for the non-native mussel species present in this region, laboratory experiments suggest that ocean warming will increase growth (Roberts 2019) and SFG (Fly and Hilbish 2013), potentially leading to changes in the distribution of these two competitor species in the region (Elliott 2008). . In the N.E. Atlantic, SFG models predict that ocean warming will cause range shifts (*M. eduilis* - Fly et al. 2015). The effect of ocean warming on phytoplankton, the primary food source for bivalves, however, differs by region (Dunstan 2018). In the Salish Sea, phytoplankton biomass may increase (Lowe et al., 2015, ~23% -Khangaonkar et al. 2019). While greater food availability might ameliorate negative effects of climate change on SFG, this ‘buffering’ effect would depend on the capacity of organisms to feed, which is a function of temperature.

We manipulated the production of a structural material to evaluate the trade-off between its production and growth and used an energetics model to evaluate the energetic cost of variable structural material production. There can be an energetic cost of many traits that exhibit phenotypic plasticity (Padilla and Savedo 2011), and our approach may be applied to other

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inducible structural traits. Examples include organisms with inducible defenses, such as herbivore-induced thorn production (Young 1987) and predator-cue induced shell thickening (Brookes and Rochette 2007). Phenotypic plasticity of structural materials can also occur as a result of environmental conditions such as wind exposure and trees, where some trees allocate energy to development and thickening of structural roots in response to wind gust direction (Nicoll and Dunn, 2000) and altered development due to wind exposure can reduce plant size and fecundity (Telewski 1998, Chehab 2008). Energetics models can include thermal performance curves and additional energy allocation 'compartments' such as energetic reserve as part of the model framework (Kitchell 1977, Kooijman 2001). Such mechanistic models that incorporate energy allocation to structural material production and other functional traits may be used to address specific research questions relating to energetic trade-offs between functional traits and organism growth in the context of environmental variability and change.

In summary, this study showed that the cost of producing byssal threads ranged from 2 to 47% of the energy budget depending on season and thread production rate, and that allocation of energy to byssus was 6 to 66% of somatic metabolic costs. Further, this study demonstrated a methodology for quantifying the costs associated with producing a structural biomaterial by manipulating its production. This general approach can be applied to other organisms with inducible biomaterial production to evaluate the energetic cost of producing these structures. Energetic constraints from decreased food availability or greater metabolic costs at greater temperatures (Bennett and Lenski, 2007) could also strengthen the trade-off between biomaterial production and growth, affecting the degree to which structural biomaterials necessary for survival are prioritized by organisms over other processes (Walker 2007, Koehl 1996). Future work demonstrating the effect of energetic limitations on functional trade-offs will be needed to increase our understanding of adaptive evolution of structural materials, and to inform improved practices for natural resource management and conservation.

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7. Supporting Information

Additional supporting information may be found in the online version of this article

Appendix S1. Length-weight relationship and optimal size (W_{opt}) data collection and parameter estimation.

Figure S1 The effect of severing treatment and byssal thread production on tissue growth

Figure S2 Sensitivity analysis of the effect of parameter and population error on SFG model output

Figure S3 Growth predictions as a function of thread production and initial tissue weight

Figure S4 Salinity and temperature during the spring and autumn experimental periods

Table S1 Energy conversion factors for *Mytilus spp.*

Table S2 Regression of tissue growth as a function of byssal thread production and initial tissue mass

Table S3 ANCOVA of the effect of severing treatment and byssal thread production on tissue growth

Table S4 Exponential relationship between byssal thread production and the proportion of cost towards byssus

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8. Figures and Tables

Table 1. Summary of one-way ANOVAs evaluating the fixed effect of byssus severing frequency on byssal thread production, shell growth, buoyant weight change, tissue growth, final gonad index, and final condition index. The autumn and spring manipulations were analyzed separately. Bold font indicates a significant effect ($p > 0.05$) and pair-wise comparisons (Tukey HSD) identified significant differences between groups.

		Thread production (# / week)				Shell growth length (mm)				Buoyant weight change (mg)			
Season	Effect	SS	Df	F val.	p	SS	Df	F val.	p	SS	Df	F val.	p
Autumn	Frequency	1181	2	64.89	**<0.001**	0.02	2	8.74	**<0.001**	0.02	2	7.09	**0.002**
	Residuals	364	40			0.06	42			0.1	42		
	Freq.	Group				Group				Group			
Tukey	Never	a				a				a			
HSD	Weekly	b				a				ab			
	Daily	c				b				b			
		Tissue growth (g)				Gonad Index (g DW/g DW)				Condition Index (g DW/g DW)			
	Effect	SS	Df	F val.	p	SS	Df	F val.	p	SS	Df	F val.	p
	Frequency	0.02	2	10.65	**<0.001**	0.01	2.00	0.35	0.70	0.03	2	0.33	0.72
	Residuals	0.05	42			0.4	40.0			1.8	42		
	Freq.	Group				Group				Group			
Tukey	Never	a				-				-			
HSD	Weekly	a				-				-			
	Daily	b				-				-			
		Thread production (# / week)				Shell growth length (mm)				Buoyant weight change (mg)			
	Effect	SS	Df	F val.	p	SS	Df	F val.	p	SS	Df	F val.	p
Spring	Frequency	934	2	123.3	**<0.001**	0.01	2	4.90	**0.01**	0.001	2	2.34	0.11
	Residuals	132	35			0.04	35			0.008	35		
	Freq.	Group				Group				Group			
Tukey	Never	a				a				-			
HSD	Weekly	b				ab				-			

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	Daily	c	b	-

		Tissue growth (g)				Gonad Index (g DW/g DW)				Condition Index (g DW/g DW)			
	Effect	SS	Df	F val.	p	SS	Df	F val.	p	SS	Df	F val.	p
	Frequency	0.01	2	5.36	**0.01**	0.00	2	0.09	0.92	0.10	2	1.84	0.17
	Residuals	0.02	35			0.3	35			1.0	35		
	Freq.	Group				Group				Group			
Tukey	Never	a				-				-			
HSD	Weekly	ab				-				-			
	Daily	b				-				-			

Table 2. Summary of parameter calculations for the Scope for Growth model. The model had five input parameters, each estimated separately for each season using constants obtained from this and previously published studies. b) SFG parameter values were derived from estimations of the energetic optimal size (W_{opt}), respiration, shape coefficient, and the relationship between wet and dry mass. Error propagation was used to estimate parameter variance from data sources. Where possible, values were estimated from the studied subpopulation, rather than using a separate set of mussels or dataset (i.e. δ).

Parameter		Unit	Season	Value	SE	Equation	Source
Input Parameter							
a'	Intake coefficient	J / (day × f × mgDW^{d})	Aut	0.90	0.26	$a'=(b*e)/(W_{opt}{}^{(d-e)*d})$	Equation from Sebens 1982
			Spr	1.76	0.55		
b	Cost coefficient	J / (day × mg DW)	Aut	0.081	0.019	$b=R\times(4.75 cal/mlO_2)$	Calculation
			Spr	0.158	0.043		
d	Intake exponent	unitless	All	0.69	0.01	Gill area = $(len^3)^d$	Jones et al. 1992 M. edulis (Van der Veer 2006 - 0.67)
e	Cost exponent	unitless	All	1			Van der Veer 2006

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C.F.	Energetic conversion factor	J / mg DW	All	21.6	1.6		Table S1
Measured values used to calculate input parameters							
W_{opt}	Energetic optimum size	g DW	All	0.72	0.06	-	Unpublished data, E. Roberts
R	Respiration	ml O2 / hr	Aut	0.073	0.017	-	Fly and Hilbish 2013 (0.429g DW in Autumn and 0.247g DW in Spring)
			Spr	0.082	0.022	-	
R_g	Respiration	ml O2 / (hr × g DW)	Aut	0.170	0.040	R_g = R / g DW	Calculation
			Spr	0.332	0.089		
δ	Volumetric mass coefficient	mg DW / (cm^3)	Aut	8.2	0.3	Mass = δ × $(\text{length})^3$	This paper
			Spr	6.8	0.2		
ratio	Conversion coefficient	mg WW / mg DW	All	3.98	0.07		This paper, separate sampling (n=100)

Table 3. Summary of parameter estimations of the cost per thread (*h*) and the food scalar (*f*) using the two-step optimization for the autumn and spring datasets.

	cost per thread (*h*; J/thread)		food scalar (*f*, Proportion)	
Season	Estimate ± SE	p	Estimate ± SE	p
Autumn	1.01 ± 0.37	**0.01**	1.42 ± 0.09	**<0.001**
Spring	1.16 ± 0.39	**0.005**	1.00 ± 0.04	**<0.001**

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Table 4. Summary of model outputs estimating energy budget allocations to producing byssus. Energy allocation to byssus as a proportion of the energy budget and as a proportion of metabolic cost (excluding reproductive tissue maintenance costs), for each of the byssal thread production treatments in the two field manipulations.

		Autumn		Spring	
	Treatment	Estimate	SE	Estimate	SE
Proportion of energy budget	Never	0.08	0.01	0.02	0.001
	Weekly	0.20	0.03	0.07	0.01
	Daily	0.47	0.04	0.23	0.02
Proportion of cost	Never	0.24	0.03	0.06	0.003
	Weekly	0.44	0.04	0.18	0.02
	Daily	0.66	0.02	0.41	0.02

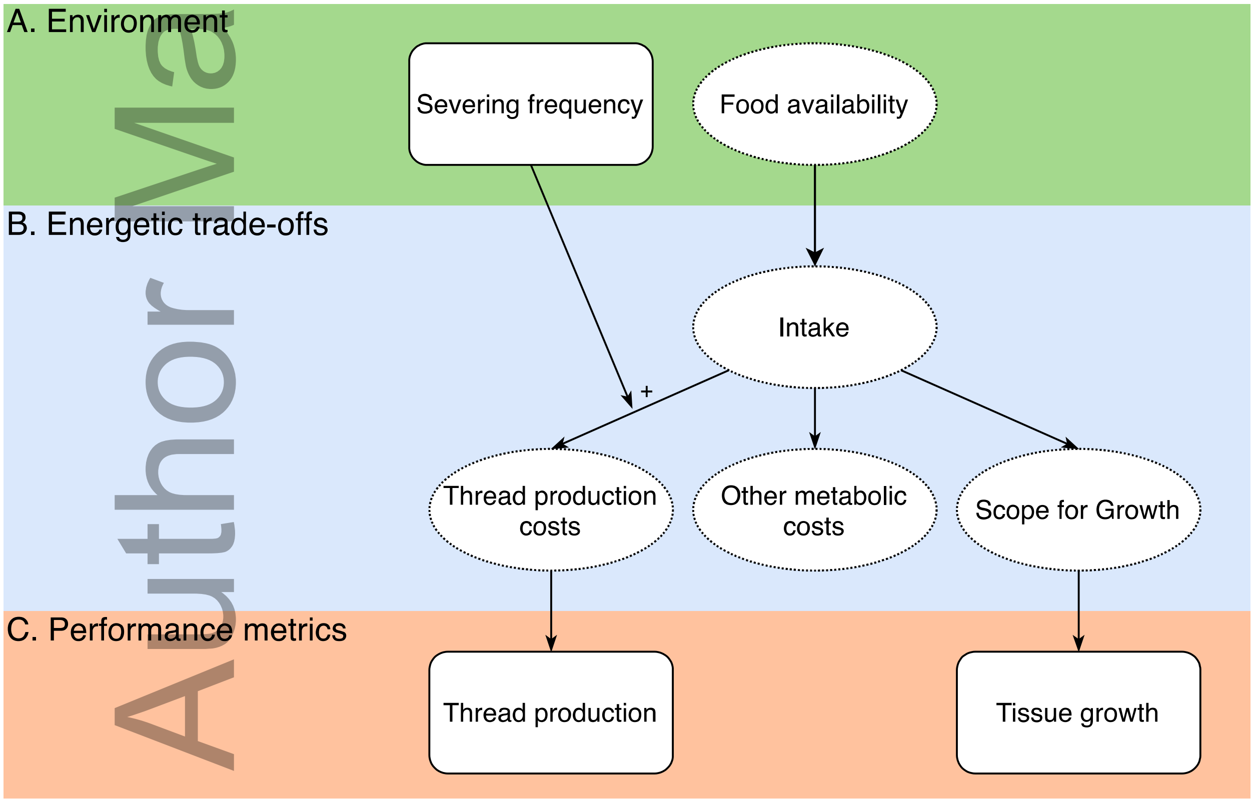

Figure 1. Schematic representation of possible energetic trade-offs between mussel byssal thread production and growth using a Scope for Growth framework. Environmental conditions (A),

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such as increased wave disturbance, predation pressure or, in the case of this study, experimental byssal thread removal by severing, can increase byssal thread production and affect other performance metrics (B, C). We hypothesized that energy allocation to byssal thread production is prioritized over tissue growth, which includes growth of new somatic and reproductive tissue. We considered tissue growth as an index of the theoretical Scope for Growth (B) since gamete production was minimal during these experiments. We used experimental observations of the relationship between thread production and growth to determine the cost of producing threads. 'Intake' indicates assimilated intake. Thread production is considered a metabolic cost separately from other metabolic costs, which includes respiration costs of somatic and reproductive tissue. Solid rectangles indicate empirical observations that were experimentally quantified, and dashed circles indicate model components. See text for details.

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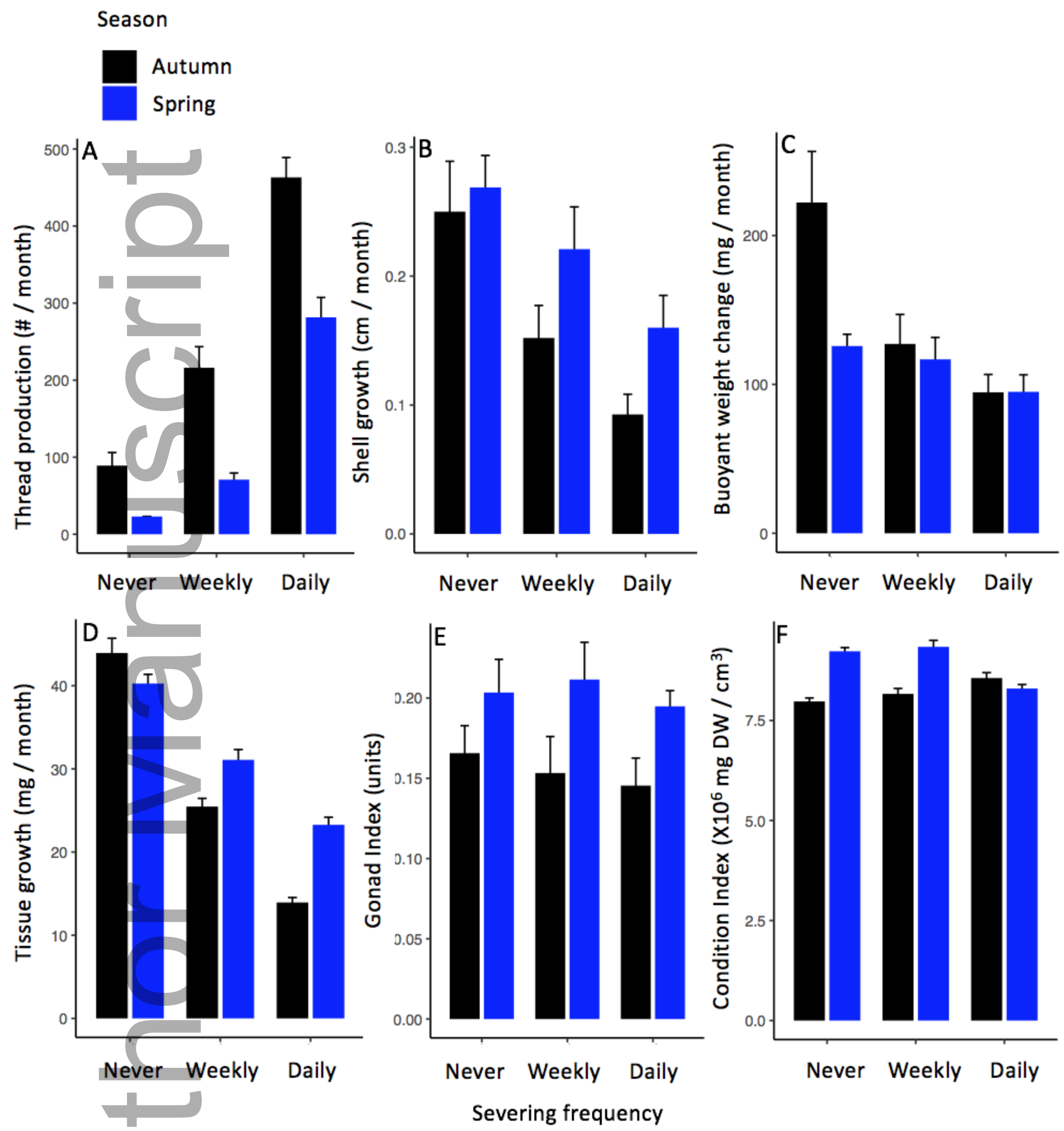

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Figure 2. Summary of byssal thread production, growth, and mussel condition across a range of byssus severing frequencies in autumn and spring. (A) Thread production, (B) shell growth, (C) buoyant weight change, (D) tissue dry weight growth, (E) final gonad index, and (F) final condition index as a function of the frequency at which the byssus was severed in autumn (black bars) and spring (blue bars; means ± SE, n = 15 in autumn, n = 11-14 in spring). The byssus was severed at a range of frequencies: once at the start of the experiment ("never"), once per week

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("weekly"), and once per day ("daily"). The change in buoyant weight is the change in weight of the living mussel, inclusive of its shell.

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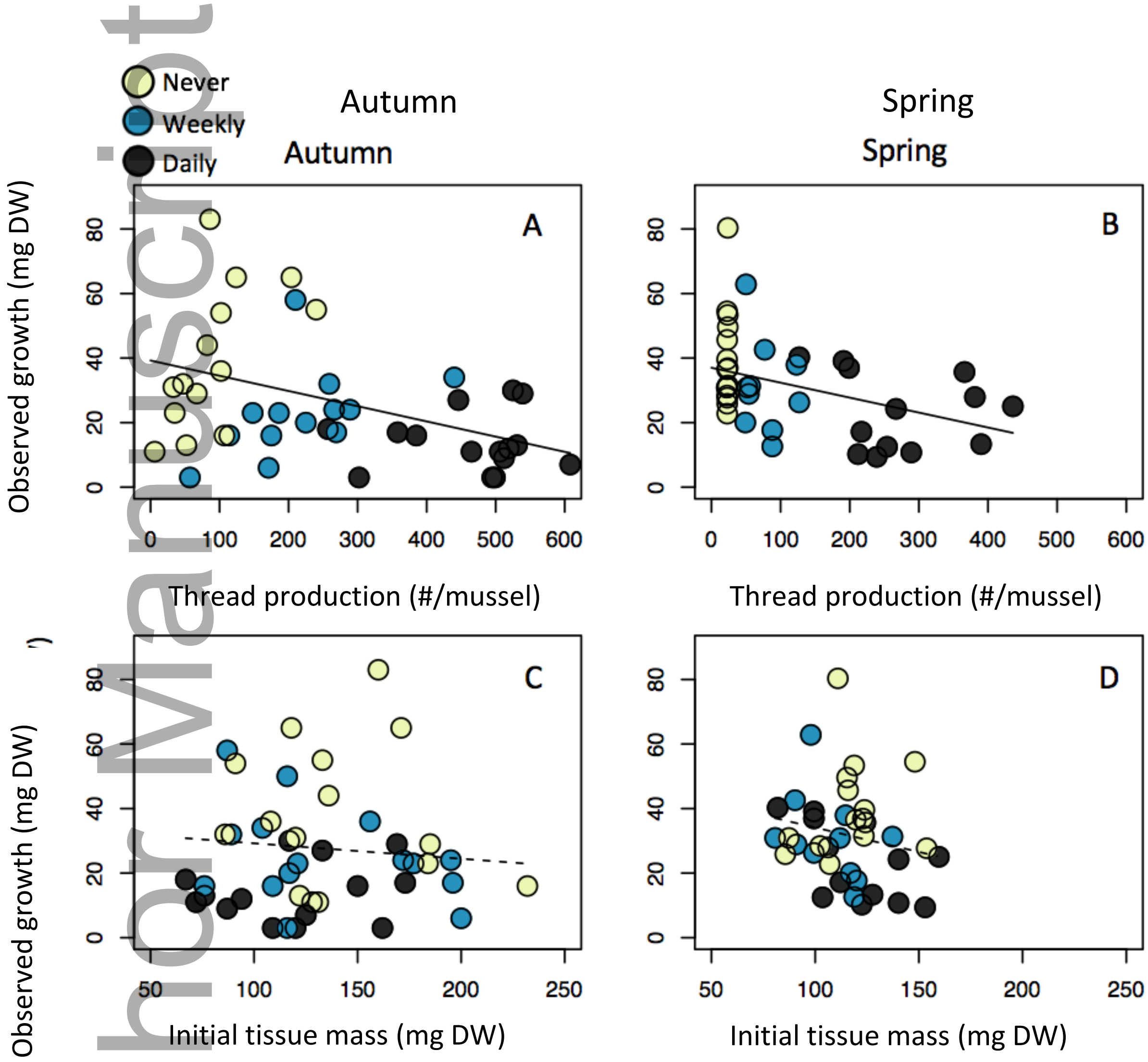

Figure 3. Mussel tissue growth as a function of thread production and initial tissue mass. Tissue growth as a function of (A, B) thread production and (C, D) estimated initial tissue mass in the autumn and spring. Symbols represent individual mussels in different severing frequency treatments (see inset for color scheme) and data were pooled across treatments for regression analyses. There was a significant negative relationship between thread production and tissue growth (A, B), but not initial tissue mass in both seasons (C, D; Table S2). Observed growth (mg DW) divided by the energetic conversion factor (C.F.) is G_{TM}.

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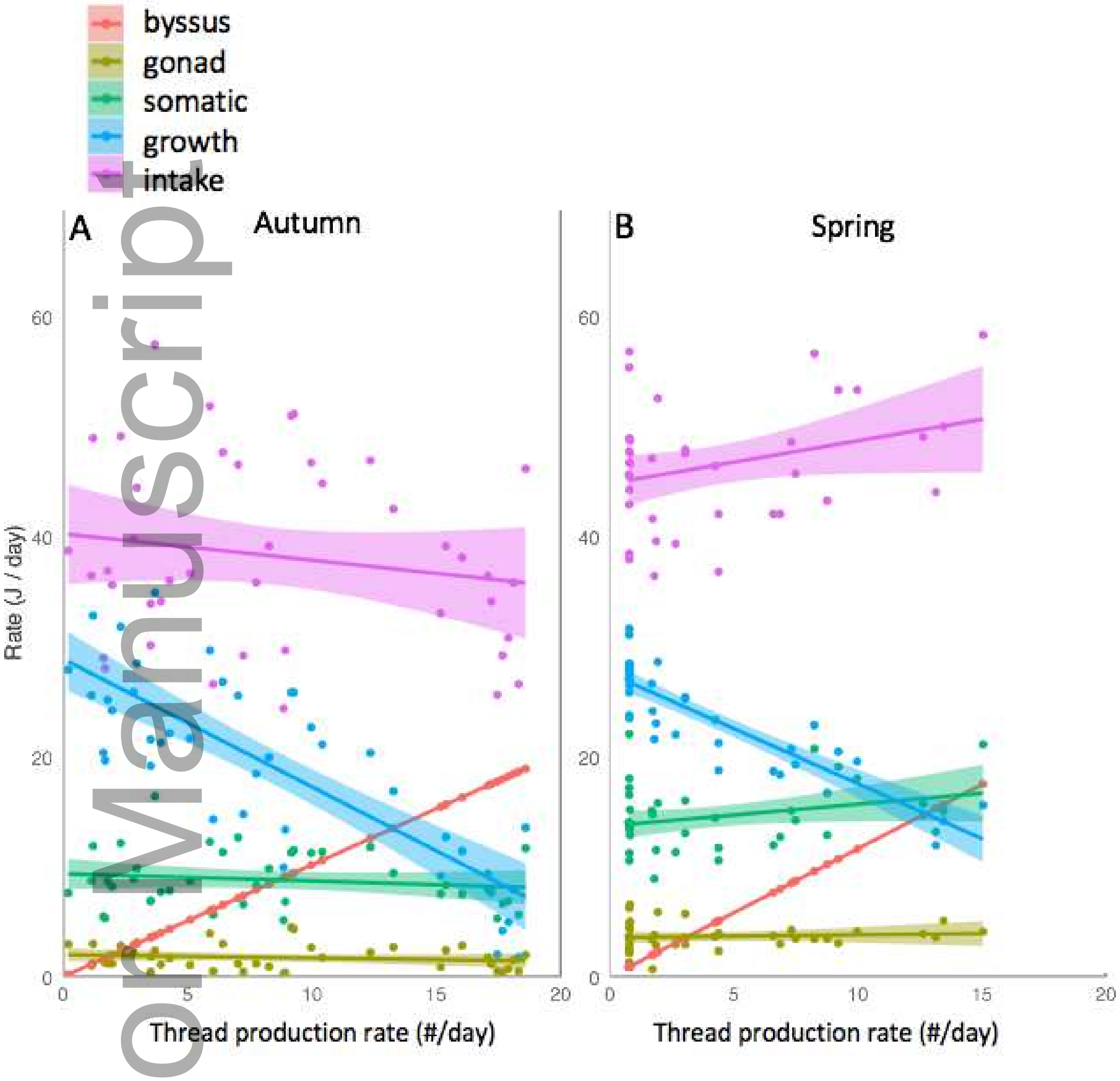

Figure 4. Model results for all components of a mussel's energy budget (J per day) as a function of byssal thread production rate (threads per day). Model results are presented for autumn (A) and spring (B) as determined by the two-step optimization. Circles represent calculated values of budget components (see inset for color scheme) for each individual; lines are linear regressions ± 95% confidence intervals. Byssus production cost does not deviate from the regression line because it is calculated as directly proportional to the thread production rate measured during the experiment. Growth (blue) represents the SFG value determined for each mussel. Somatic (green) is the maintenance cost for somatic tissue, and Gonad (yellow) is the maintenance cost for gonad during this time period.

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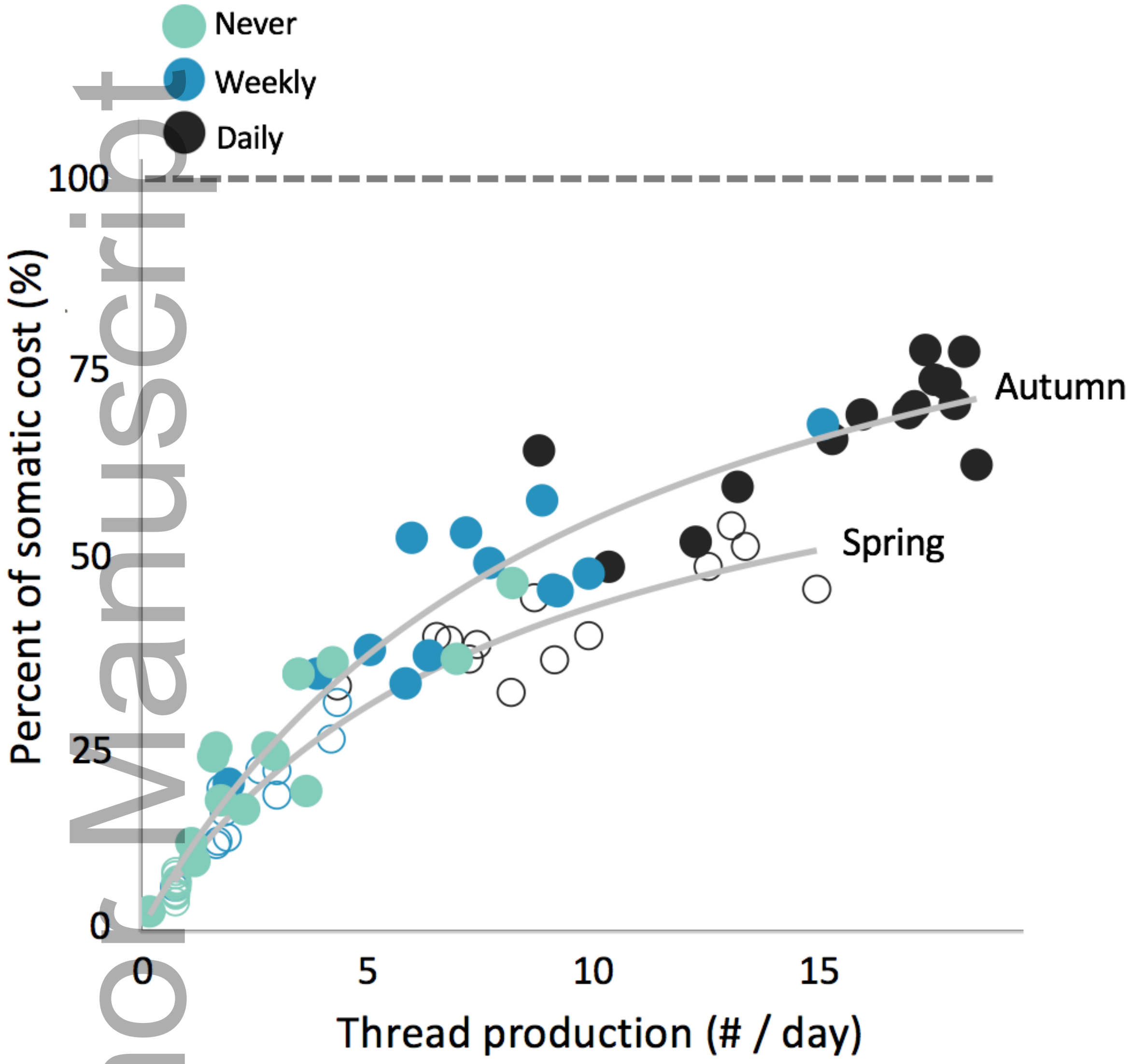

Figure 5. Energy allocation towards byssal threads, expressed as a proportion of metabolic costs of tissue maintenance (excluding reproductive costs), as a function of thread production in the autumn (closed circles) and spring (open circles). Symbol colors represent the frequency of severing in the treatment. Each curve is an exponential fit (proportion of cost $= V_{\max}(1 - e^{\frac{-N_{Th}}{\tau}})$, Table S4).

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Table 1. Summary of one-way ANOVAs evaluating the fixed effect of byssus severing frequency on byssal thread production, shell growth, buoyant weight change, tissue growth, final gonad index, and final condition index. The autumn and spring manipulations were analyzed separately. Bold font indicates a significant effect (p > 0.05) and pair-wise comparisons (Tukey HSD) identified significant differences between groups.

		Thread production (# / week)				Shell growth length (mm)				Buoyant weight change (mg)			
Season	Effect	SS	Df	F val.	p	SS	Df	F val.	p	SS	Df	F val.	p
Autumn	Frequency	1181	2	64.89	**<0.001**	0.02	2	8.74	**<0.001**	0.02	2	7.09	**0.002**
	Residuals	364	40			0.06	42			0.1	42		
	Freq.	Group				Group				Group			
Tukey	Never	a				a				a			
HSD	Weekly	b				a				ab			
	Daily	c				b				b			

		Tissue growth (g)				Gonad Index (g DW/g DW)				Condition Index (g DW/g DW)			
	Effect	SS	Df	F val.	p	SS	Df	F val.	p	SS	Df	F val.	p
	Frequency	0.02	2	10.65	**<0.001**	0.01	2.00	0.35	0.70	0.03	2	0.33	0.72
	Residuals	0.05	42			0.4	40.0			1.8	42		
	Freq.	Group				Group				Group			
Tukey	Never	a				-				-			
HSD	Weekly	a				-				-			
	Daily	b				-				-			

		Thread production (# / week)				Shell growth length (mm)				Buoyant weight change (mg)			
	Effect	SS	Df	F val.	p	SS	Df	F val.	p	SS	Df	F val.	p
Spring	Frequency	934	2	123.3	**<0.001**	0.01	2	4.90	**0.01**	0.001	2	2.34	0.11
	Residuals	132	35			0.04	35			0.008	35		
	Freq.	Group				Group				Group			
Tukey	Never	a				a				-			
HSD	Weekly	b				ab				-			

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	Daily	c	b	-

		Tissue growth (g)				Gonad Index (g DW/g DW)				Condition Index (g DW/g DW)			
	Effect	SS	Df	F val.	p	SS	Df	F val.	p	SS	Df	F val.	p
	Frequency	0.01	2	5.36	**0.01**	0.00	2	0.09	0.92	0.10	2	1.84	0.17
	Residuals	0.02	35			0.3	35			1.0	35		
	Freq.	Group				Group				Group			
Tukey	Never	a				-				-			
HSD	Weekly	ab				-				-			

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Table 2. Summary of parameter calculations for the Scope for Growth model. The model had five input parameters, each estimated separately for each season using constants obtained from this and previously published studies. SFG parameter values were derived from estimations of the energetic optimal size (W_{opt}), respiration, shape coefficient, and the relationship between wet and dry mass. Error propagation was used to estimate parameter variance from data sources. Where possible, values were estimated from the studied subpopulation, rather than using a separate set of mussels or dataset (i.e. δ).

Parameter		**Unit**	**Season**	**Value**	**SE**	**Equation**	**Source**
Input Parameter							
a'	Intake coefficient	J / (day × f × mgDW^{d})	Aut	0.90	0.26	$a'=(b*e)/(W_{\text{opt}}{}^{(d-e)*d})$	Equation from Sebens 1982
			Spr	1.76	0.55		
b	Cost coefficient	J / (day × mg DW)	Aut	0.081	0.019	$b=R\times(4.75\text{cal/ml}O_2)$	Calculation
			Spr	0.158	0.043		
d	Intake exponent	unitless	All	0.69	0.01	Gill area = $(\text{len}^3)^d$	Jones et al. 1992 M. edulis (Van der Veer 2006 - 0.67)
e	Cost exponent	unitless	All	1			Van der Veer 2006
C.F.	Energetic conversion factor	J / mg DW	All	21.6	1.6		Table S1
Measured values used to calculate input parameters							
W_{opt}	Energetic optimum size	g DW	All	0.72	0.06	-	Unpublished data, E. Roberts
R	Respiration	ml O2 / hr	Aut	0.073	0.017	-	Fly and Hilbish 2013 (0.429g DW in Autumn and
			Spr	0.082	0.022	-	

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							0.247g DW in Spring)
R_g	Respiration	ml O2 / (hr × g DW)	Aut	0.170	0.040	R_g = R / g DW	Calculation
			Spr	0.332	0.089		
δ	Volumetric mass coefficient	mg DW / (cm^3)	Aut	8.2	0.3	Mass = δ × $(\text{length})^3$	This paper
			Spr	6.8	0.2		
ratio	Conversion coefficient	mg WW / mg DW	All	3.98	0.07		This paper, separate sampling (n=100)

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Table 3. Summary of parameter estimations of the cost per thread (*h*) and the food scalar (*f*) using the two-step optimization for the autumn and spring datasets.

	cost per thread (*h*; J/thread)		food scalar (*f*, Proportion)	
Season	Estimate ± SE	p	Estimate ± SE	p
Autumn	1.01 ± 0.37	**0.01**	1.42 ± 0.09	**<0.001**
Spring	1.16 ± 0.39	**0.005**	1.00 ± 0.04	**<0.001**

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Table 4. Summary of model outputs estimating energy budget allocations to producing byssus. Energy allocation to byssus as a proportion of the energy budget and as a proportion of metabolic cost (excluding reproductive tissue maintenance costs), for each of the byssal thread production treatments in the two field manipulations.

		Autumn		Spring	
	Treatment	Estimate	SE	Estimate	SE
Proportion of energy budget	Never	0.08	0.01	0.02	0.001
	Weekly	0.20	0.03	0.07	0.01
	Daily	0.47	0.04	0.23	0.02
Proportion of cost	Never	0.24	0.03	0.06	0.003
	Weekly	0.44	0.04	0.18	0.02
	Daily	0.66	0.02	0.41	0.02

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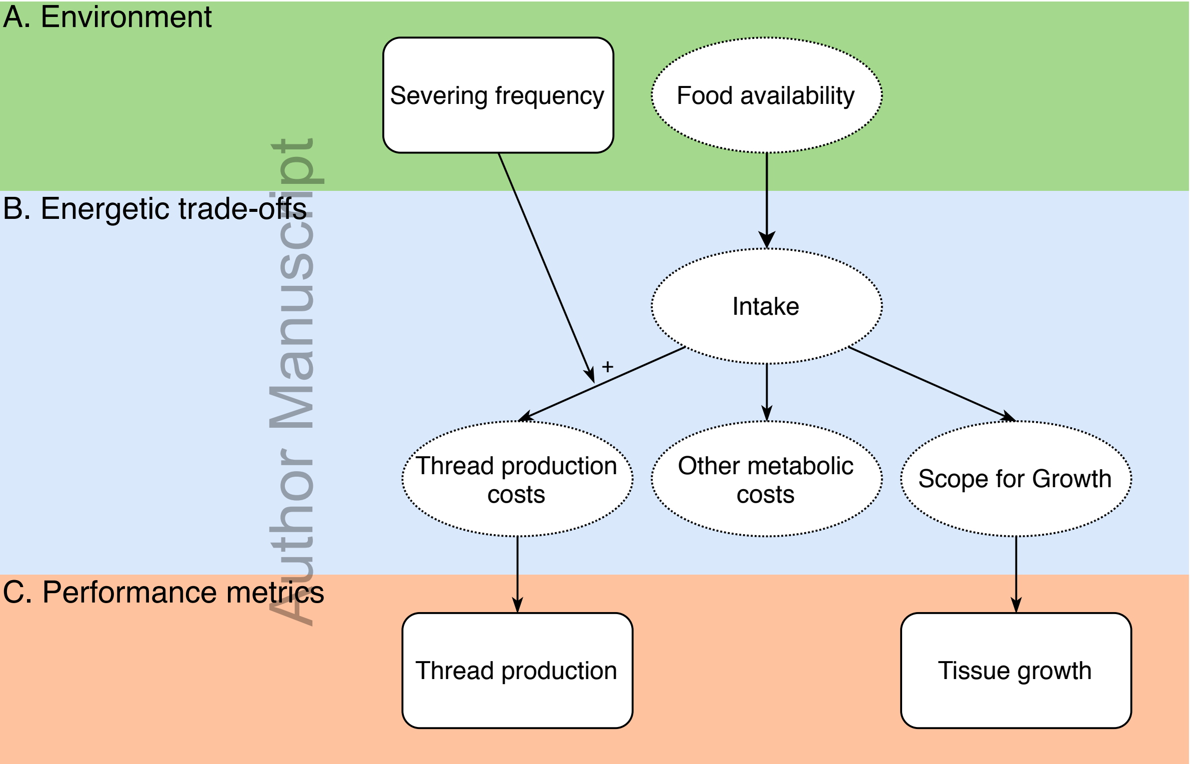

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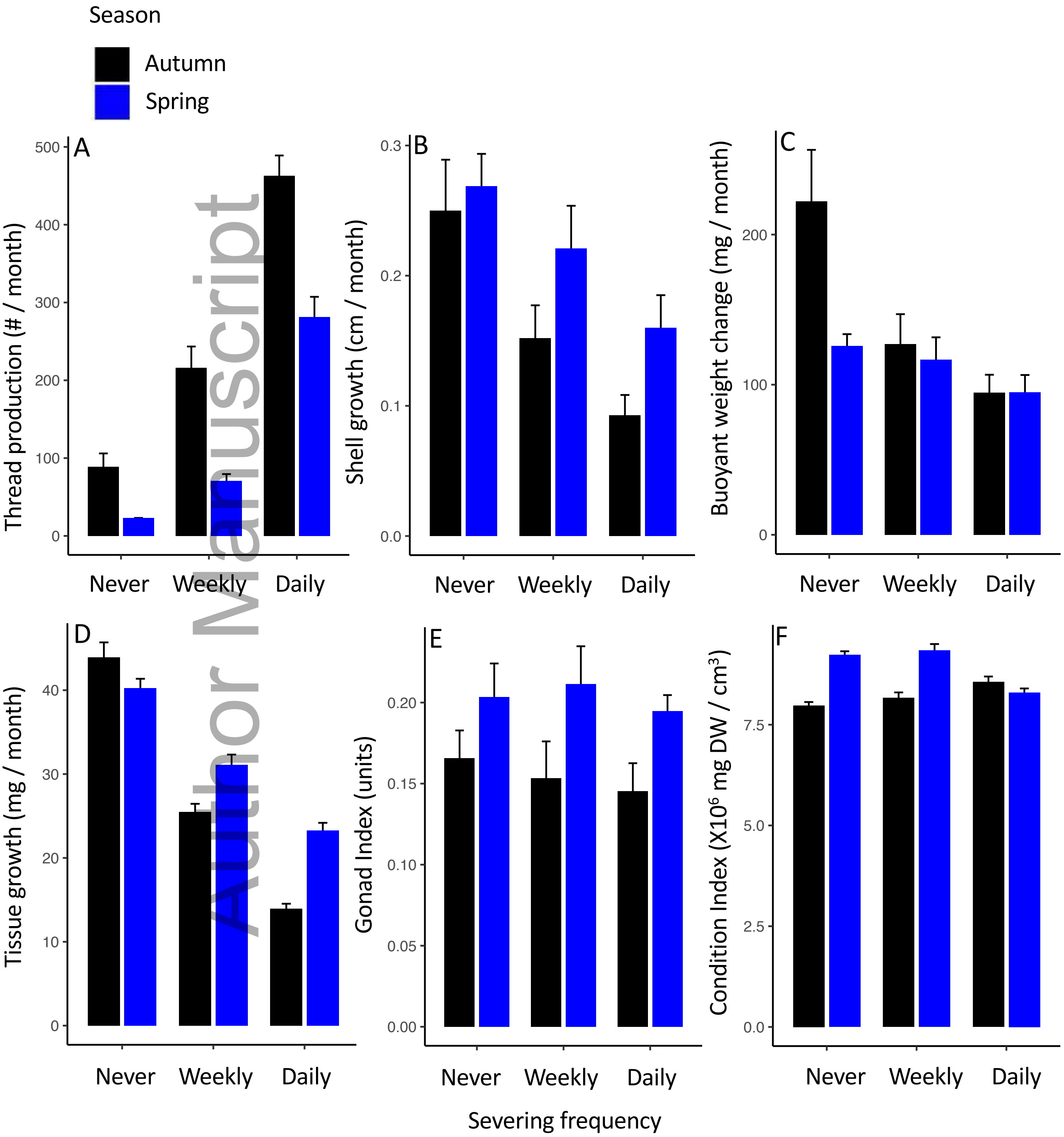

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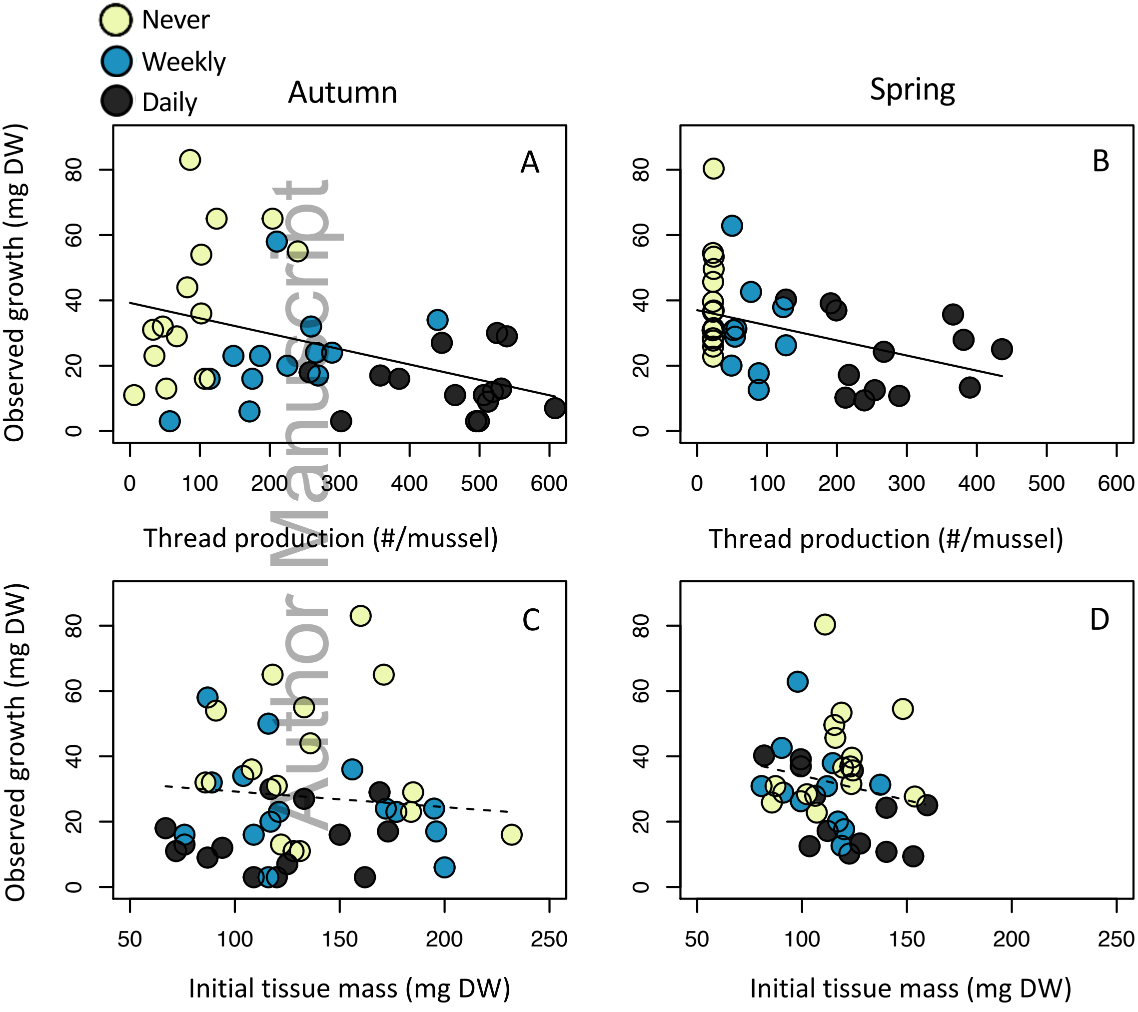

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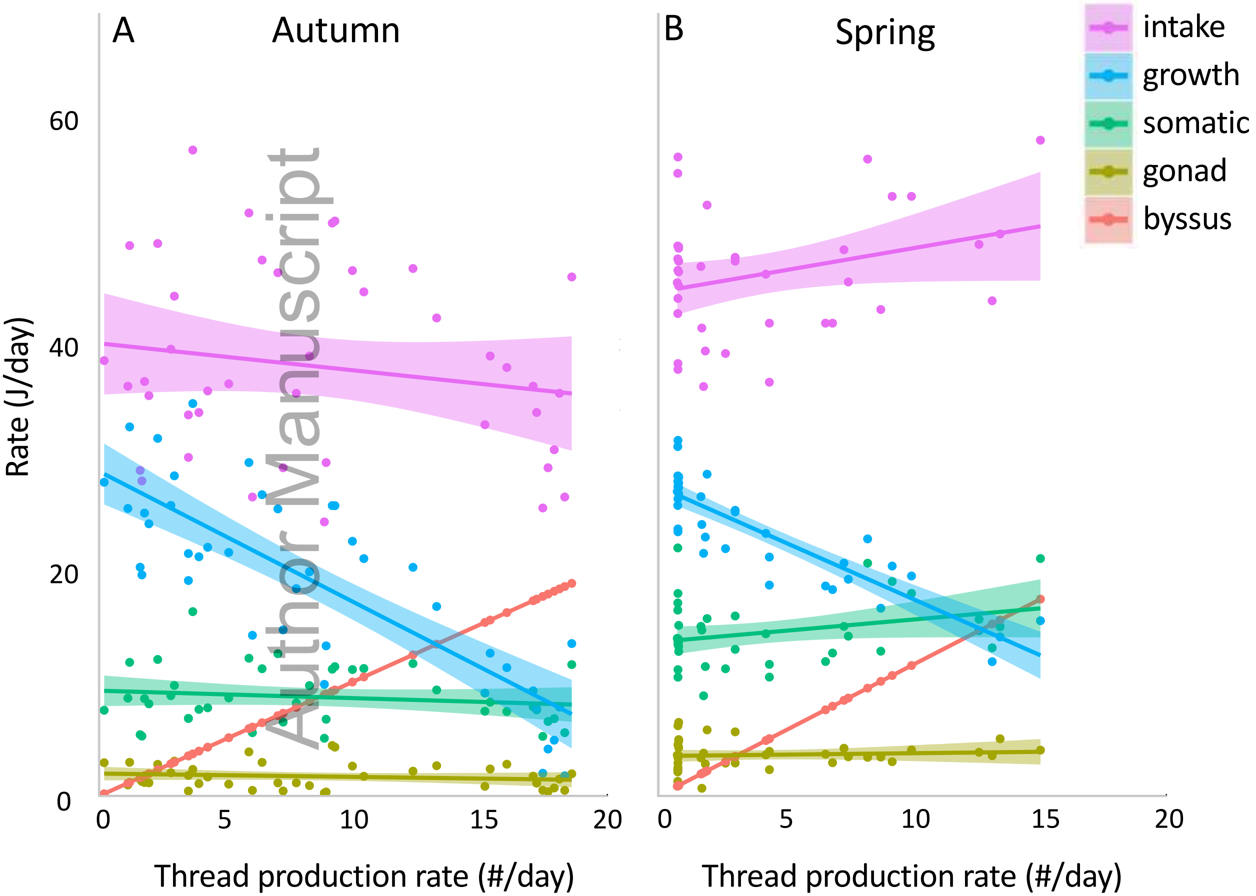

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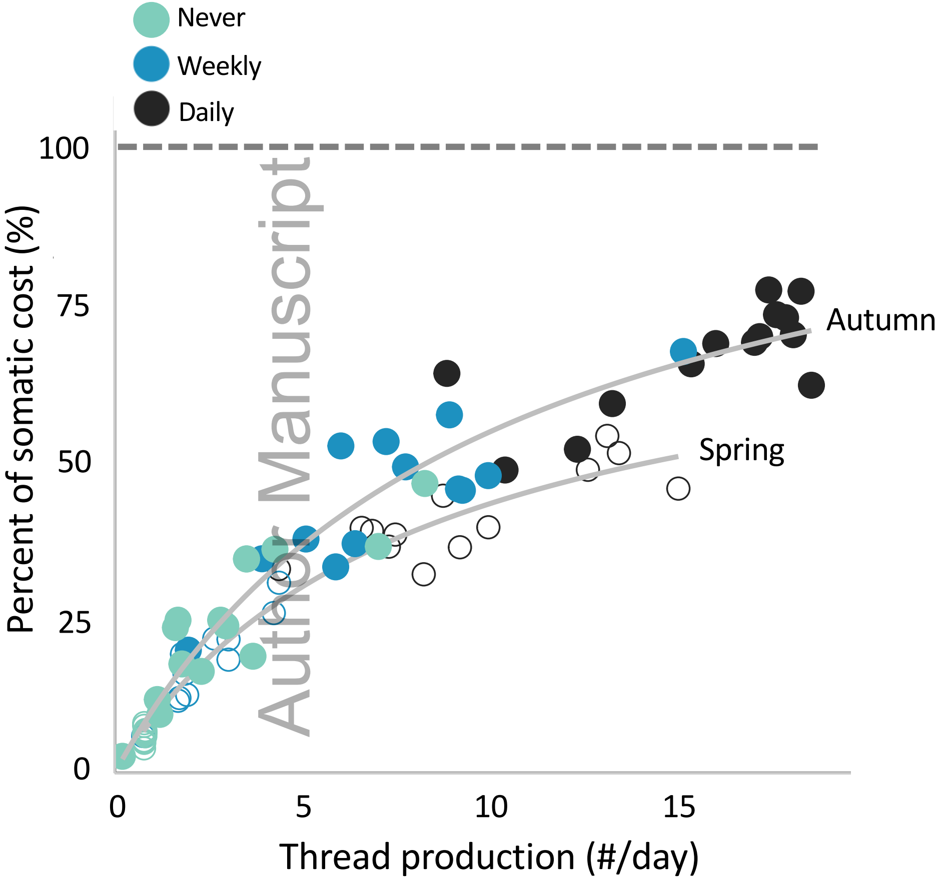

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